A prospective comparative study of five year old children (and their families) born after Intracytoplasmic Sperm Injection, conventional in-vitro fertilisation or natural conception; and other studies of child/family outcome after in-vitro fertilisation techniques.

Dr Catherine Peters, MBChB, MRCPCH

Submitted for consideration of a Doctorate of Medicine (MD)

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

Abstract

Objectives

- To assess the extent to which exposure to Intracytoplasmic Sperm Injection (ICSI) is associated with significant health, developmental and psychosocial adjustment outcomes.
- To investigate the incidence of assisted conception in children with Beckwith-Wiedemann syndrome.

Methods

- A population case-control study of 510 school age children (and their families) born after ICSI (n=189), conventional in-vitro fertilisation (IVF) (n=158) or natural conception (n=163). Outcome measures included:
  - A full physical examination of the child which included enumeration of physical abnormalities and an assessment of general health.
  - An assessment of childhood IQ using the Weschler Preschool and Primary Scale of Intelligence (WPPSI)
  - An assessment of gross and fine motor skills using the WPPSI and McCarthy Motor Skills tests
  - Questionnaires to assess of parent – child relationships
- A survey of parental attitudes towards disclosing the method of conception to their IVF children
- A survey of 160 members of the Beckwith-Wiedemann Syndrome (BWS) support group enquiring about conception

Results

- There was no difference between conception groups for overall physical health or fine/gross motor difficulties
- There was evidence of an increase in congenital abnormalities in the assisted reproduction groups.
- Parent-child relationships were similar between groups

- The majority of ICSI / IVF parents wish to disclose the method of conception to their child.
- There is an increased likelihood of children with BWS being conceived after IVF compared to the general population

Conclusion

- The studies in this thesis are reassuring, in terms of physical and neurodevelopmental health of ICSI children aged 4-5 years and their family relationships.
- The increase in congenital abnormalities after IVF/ICSI requires further study.
- Families of assisted conception children wish to disclose conception method to their child but require more support.
- There is evidence of an increased risk of BWS in children conceived after assisted reproduction.

Table of Contents

Page

Page

Page

Page

List of illustrations

List of figures

List of tables

Page

CHAPTER 1: INTRODUCTION

1.1 Historical background

In the UK, it is estimated that approximately 1 in 6 couples are subfertile (Hull *et al.* 1985). The incidence of subfertility appears to be increasing and the reasons for this are mainly unexplained. Couples are choosing to defer parenthood, often waiting until careers are established, and starting families later in life than previous generations. The average age for women to first give birth is now 27 compared with 24 in 1970 (Lansac 1995; UK Office of National Statistics).

The problem of infertility is not new, but the rapid advance in assisted conception technologies over the past 20 years has changed our response to it. The Old Testament gives several reports of infertile or "barren" women, the first example being Sarah, wife of Abraham who chose surrogacy in order to create a family (*Genesis 16v1-2*). Old texts from the ancient Egyptians, Greeks and Romans describe medicinal and herbal cures for female infertility (Inhorn 1994).

However, there are few biblical or ancient examples of male infertility and until relatively recently failure to produce children was regarded as a female failure. In the early nineteenth century, barrenness in women was seen as a defect of mind as well as body. Although male infertility was recognised, it was seldom studied until the 20th century. In the 1950's, the apparent decline in male fertility in the USA was argued to be a result of the exposure of men to hazardous chemicals and radioactive substances in war (Marsh and Ronner 1996).

There appear to be differing gender reactions to learning about infertility. For women the central aspect is often an intense desire to have a child whereas for men the fulfilment of the male and social role is central (Gibson *et al.* 2000a; Hjelmstedt *et al.* 1999). Parents of assisted conception families have reported that, on learning of their infertility, reactions included despair, isolation, envy of others and grief. Men's reactions ranged from devastation to rage. Some infertile partners also reported that they had told their partners to leave them and find an alternative mate (McWhinnie 1996)

It therefore seems important that family relationships as well as child physical development and behaviour are studied when assessing outcomes of infertility treatments. Advances in science and technology in the area of assisted fertilization are rapid but there has been little evaluation of each process before new techniques are introduced. No-one knows what the long-term outcome for the current generation of children born after assisted conception will be and what the implications are for their children. Louise Brown, the first baby born after in-vitro fertilization is 26 this year and as more IVF children reach their twenties, we may be able to see if there is any impact on the fertility of these individuals.

1.2 Early development of assisted conception techniques

Artificial insemination became increasingly used from the 1930's in the USA, but when Rock and Menkin first reported in vitro fertilisation of human eggs in *Science* in 1944 there was intense public debate and, in many quarters, moral outrage.

Work on human in-vitro fertilization (IVF) continued, but it was over 30 years later in the UK that a successful pregnancy was achieved. Steptoe and Edwards reported the birth of the first IVF baby in a letter to the Lancet in 1978 (Steptoe and Edwards 1978). Louise Brown had been born at 38 weeks and 5 days on the 25th July 1978. Since this time it is estimated that over 60,000 children (Venn 2004) have been born after IVF techniques in the UK and more than a million worldwide. The Human Embryology and Fertilization Authority (HFEA) was set up in 1991 to monitor and collect data from IVF clinics. Prior to 1991 data was held by individual clinics. It is estimated that for the years 1995-1999 inclusive, 1.6% of births in England and Wales were after IVF (Maher 2002). This is likely to increase as IVF techniques become more accessible. The UK Secretary for Health announced in February 2004 that all areas of the UK must provide at least one cycle of IVF free on the NHS by April 2005, with a longer term aim to provide at least 3 free cycles (DoH 2004).

A 'conventional' IVF cycle firstly involves hormonally induced superovulation followed by the retrieval of oocytes under ultrasound guidance. Oocytes are then transferred into a droplet of sperm suspension containing approximately 100,000

sperm per ml. The inseminated oocytes are incubated for up to 20 hours and are then dissected to assess fertilisation. Coronal and cumulus cells are removed and the presence of two pronuclei and two polar bodies indicate successful fertilisation. Cleaved embryos are then transferred to the uterus 2-3 days after fertilisation. In some instances the embryo is transferred as a blastocyst at 5 days. This is thought to enhance implantation potential (Elder and Dale 2000).

If a large number of oocytes are fertilised it is possible to cryopreserve those embryos not chosen for transfer on this occasion. This technique allows couples to have their embryos "frozen", typically at the pronucleate stage, until a date in the future when they may wish to try for another pregnancy. It has the benefit of allowing women to minimize the number of ovarian stimulation cycles and to allow fewer eggs to be implanted, thus minimising the risk of multiple births. It is also used if the mother develops severe ovarian hyperstimulation syndrome which can be worsened by pregnancy. The oocytes are fertilized and the embryos frozen until such a point that the mothers has recovered from the ovarian hyperstimulation syndrome and implantation can result in a safer pregnancy.

Although successful, these IVF techniques were unable to overcome the problems of male subfertility often associated with oligozoospermia and abnormal sperm. In addition there are instances where sperm are unable to penetrate the outer layers of the oocyte cell membranes. In these cases other techniques were needed.

In humans, embryonic implantation after invasive assisted fertilisation of the oocyte was first achieved using a method known as partial zona dissection (PZD) (Cohen *et al.* 1988). After preparing oocytes by removing the cumulus and corona cells and shrinking the cytoplasm, a small glass needle is forced through the zona pellucida prising it open. The oocytes are then washed and inseminated. In this first report by Cohen, four women had embryos replaced and two became pregnant with twins.

The subzonal injection of sperm (SUZI/SZI) was also developed around this time. Using this method, the oocyte is directly punctured by a micropipette and several

motile sperm are introduced into the perivitelline space. Ng et al reported a pregnancy following this technique in October 1988 (Ng *et al.* 1988).
In 1992, four pregnancies were reported in the Lancet (Palermo *et al.* 1992) after the use of intracytoplasmic sperm injection of the oocyte (ICSI). This method involved the aspiration of a single spermatozoon and its injection directly into an oocyte (see illustration 1.1). The mothers involved in this report had had failed treatments with conventional IVF or SUZI prior to this new technique. The outcome was two singleton pregnancies, one twin pregnancy and one preclinical abortion. Subsequently, four healthy children were delivered.

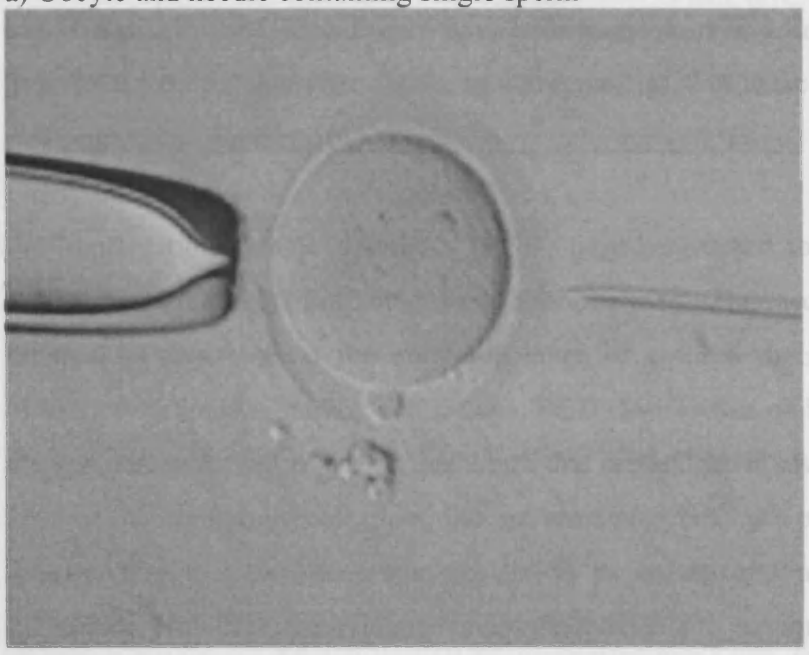
Palermo and Van Steirteghem demonstrated the effectiveness of ICSI in patients that had failed conventional IVF or SUZI or were not accepted for IVF treatment because of poor sperm parameters (Palermo *et al.* 1993; Van Steirteghem *et al.* 1993b). Van Steirteghem subsequently reported much higher fertilization rates after ICSI when compared to SUZI (72% v 4%) using sibling oocytes from 11 patients (Van Steirteghem *et al.* 1993a). Although the numbers involved were small, the results were convincing enough for the clinic to abandon SUZI as a technique. ICSI has now become the treatment of choice for male infertility and/or failed IVF.

These methods of bypassing the natural selection processes of sperm fertilisation have moved further with the development of microsurgical epididymal sperm extraction and testicular sperm extraction to be used to fertilize the oocyte. Both these techniques have resulted in high fertilization rates (Silber *et al.* 1995; Tournaye *et al.* 1994). Perhaps unsurprisingly, these methods of sperm extraction have been found to be much more successful with ICSI, and the direct injection of sperm, rather than conventional IVF which relies on good sperm quality / function for fertilisation (Silber *et al.* 1994).

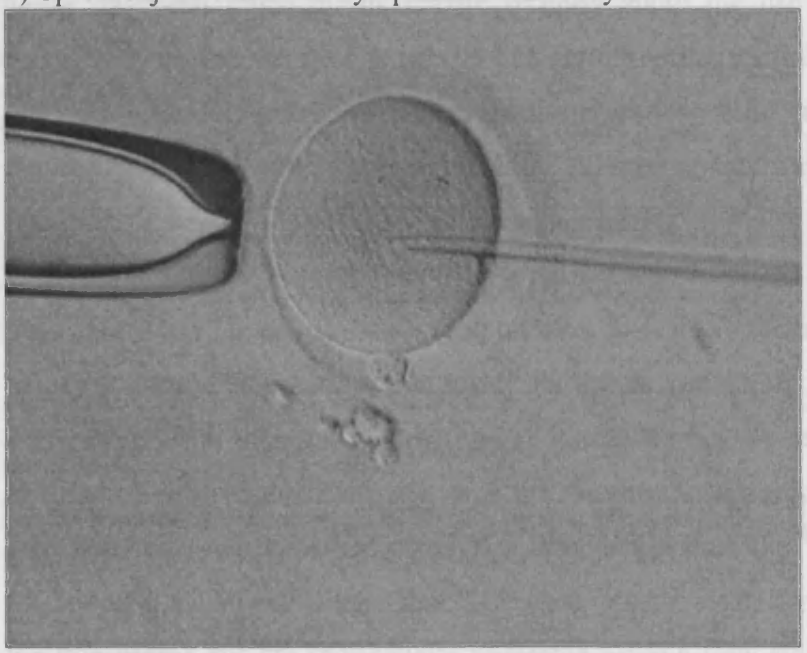
Devroey et al have also demonstrated that in men previously considered sterile, with azoospermia and/or testicular failure with severe spermatogenic defects, it is still often possible to find a single tiny point of spermatogenesis in the testicle (Devroey *et al.* 1995). A spermatozoon, even if immotile, could then be obtained and used to fertilize an oocyte using ICSI. This approach has resulted in successful pregnancies.

Illustration 1.1 Intracytoplamic sperm injection

a) Oocyte and needle containing single sperm



b) Sperm injected into the cytoplasm of the oocyte



Images provided by Dr M Bonduelle, Centre for medical genetics, Dutch-speaking Brussels Free University, Brussels, Belgium

More recently, spermatids have been injected into oocytes and fertilisation has thus occurred without full maturation of spermatozoa beforehand. This procedure has been banned in the UK and there have been some worrying initial outcome studies. Zech et al reported that after obtaining 4 pregnancies, 2 of these had major congenital malformations (Zech *et al.* 2000).

Pre-implantation genetic diagnosis (PGD) may be offered to couples in order to screen for life-threatening or severe abnormalities. This technique involves the removal of two cells at the eight-cell stage of embryo division. These cells are examined for known genetic aberrations. PGD ensures that embryos with the known genetic defect are not replaced. Although this method involves the removal of up to 25% of the embryonic cell mass, the six remaining cells are pluripotential and it is thought that they will continue to divide as before and accommodate for the deficiency. However, the evidence is not yet available to support this assumption and the technique is banned in some countries.

There has been much public debate recently about the use of this technique to specifically choose the genetic profile of a child in order for this child to donate stem cells and possibly other tissue to relatives with specific medical conditions ie thalassaemia major. The issue of selecting children in this way has led to concerns that this is a step closer to achieving "designer babies".

This fear has been further compounded by new techniques which allow embryologists to select the sex of the child. Sperm containing X chromosomes have 2.8% more DNA than those containing Y chromosomes. Sperm DNA can therefore be marked with fluorescent dye and the X chromosomes with more DNA will fluoresce more when passed through a laser beam than the Y chromosomes. The MicroSort® technique utilises this difference to separate sperm. Sperm marked with the fluorescent dye are individually separated using a flow cytometry. The X and Y sperm are then given opposing electrical charges and separated by electromagnetic plates.

The pace of change in this field continues and some scientists are already determined to clone humans and are working on this despite objections from most scientific and public bodies. The use of genetic material from cells other than gametes for fertilization of the oocyte is also being debated. Without studies fully confirming the safety of techniques already used, it is hard to see how these methods can be scientifically or ethically justified.

1.3 Sociodemographic profile of IVF parents – an overview of the literature

1.3.1 Social class

Most studies try to match social class and there is a tendency for IVF families to be from higher than social class 3 (Bowen *et al.* 1998; Sutcliffe *et al.* 2001). In the past, most UK families have had to fund IVF treatment themselves and this may preclude many poorer families from seeking this treatment. In many other countries IVF still requires private funding.

1.3.2 Ethnicity

There are many studies of IVF children in caucasian families. In the UK, there have been some studies into IVF treatment in the asian population. An initial small study (Mahmud *et al.* 1995) found that British women of Indian descent responded less well to ovarian stimulation and had a higher miscarriage rate than white British women and were therefore less successful in becoming pregnant after IVF. A further larger study in 1999 (Lashen *et al.* 1999) of UK born women of Indian and white descent found no difference in ovarian stimulation and IVF outcome. The reason for the discrepancy in study outcomes is not clear, but the latter study was larger. Both studies found that the duration of infertility in the women of Indian descent was longer before seeking treatment. This may be explained by the differences in the community cultures.

Sharara et al evaluated the differences in IVF outcome between black and white women (Sharara and McClamrock 2000). Black women represented 28% of the women treated at that clinic, but the rate of infertility within the black population is not established. The causes of infertility were found to be different, with an increase in tubal factor problems in black women and an increase in endometriosis and male

infertility in the white population. The black women were also found to have higher body mass indices (BMI) and longer duration of infertility. They also had a lower implantation rate, a higher rate of pregnancy loss and required more aggressive ovarian stimulation. Overall the black women had a 2.6 fold decreased odds of becoming pregnant.

1.3.3 Maternal age

Mothers of IVF children are often older then other mothers (Leslie *et al.* 1998; Tan *et al.* 1992; Klemetti *et al.* 2002). Kane in 1967 described a gradual increase in risk for low birth weight and perinatal mortality in primiparous women with age from 25 to 40 years (Kane 1967). Additional early studies of the influence of maternal age on antenatal and intrapartum outcomes suggested that the perinatal mortality rate increases with maternal age and this rate is doubled in women over the age of 40 years (Biggs 1973).

Mothers in this study by Biggs had a high rate of multiparity and there was a higher rate of delivery by caesarian section in this group. Booth and Williams also studied primigravida women over the age of 35 and again found a higher rate of perinatal mortality and caesarian section births than in primigravidae women under the age of 34 (Booth and Williams 1964).

These early studies did not take into account the mother's previous history of infertility or spontaneous abortions. It is possible therefore that the mothers in these studies were older because of obstetric difficulties and these mothers might not be comparable with older mothers in more recent studies who have chosen to delay childbearing.

However, a Swedish report in 1984 (Forman *et al.* 1984) found that there was an increase in low birth weight and /or preterm deliveries with increasing maternal age and that this increase was present regardless of reproductive history. A further Swedish study (Cnattingius *et al.* 1992) reported similar findings and suggested that there was a 40% increased risk of late fetal death in women over the age of 30 when

compared to a group aged 20-24 after adjustment for maternal complications and risk factors, including a prior history of infertility.

In contrast, Barkan and Braken studied the effect of increased maternal age on white primiparous women and analysed the outcomes according to past obstetric histories (Barkan and Bracken 1987). They found that women over the age of 30 without a previous history of miscarriage or infertility were not at an increased risk of preterm or low birth weight deliveries.

Berkowitz et al studied 3917 mothers over the age of 20 years and found that older mothers were more likely to have antenatal and intrapartum complications, caesarian sections and have infants admitted to a neonatal unit (Berkowitz *et al.* 1990). They did not find an increase in perinatal mortality, preterm delivery or small for gestational age infants. This study included only private patients with a higher socio-economic status and level of education than many other groups studied.

Cohen and Freidman also suggested that there was an increase in dysfunctional (protracted or arrested) labour with increasing maternal age and they have hypothesised that this may be due to decreasing myometrial efficiency with age (Cohen *et al.* 1980).

It is also well established that trisomies, particularly trisomy 21 have an increasing incidence with maternal age (Hassold *et al.* 1996).

1.3.4 Paternal age

Fathers of IVF and ICSI children also tend to be older than other fathers (Leslie *et al.* 1998). There is a slow decrease in motility, morphology and numbers of spermatozoa with age. This becomes more obvious over the age of 55 (Lansac 1995). A history of torsion and trauma to the testes increases the risk of azoospermia by 5.4. Toxins, heat, medication and sexually transmitted diseases can all adversely influence male fertility (Lansac 1995).

The evidence that increasing paternal age has an effect on the incidence of trisomies is conflicting (Griffin *et al.* 1995). It is only in the last ten years that it has been possible to differentiate between maternal and paternally derived trisomies. This is mainly because 80-90% of trisomies are known to be maternal in origin and studies of paternally derived trisomies are small in number and have limited power (Griffin *et al.* 1995). However, the study by Griffin et al found that there was a small increase in sex chromosome disomy in sperm with advancing paternal age.

McIntosh et al also suggested an increase in major birth defects with increasing paternal age (McIntosh *et al.* 1995). It has been hypothesised that this is due to "copy error" where errors occur during the repeated cell replications in spermatogenesis. Paternal age is known to have an effect on the incidence of Apert's syndrome (Glaser *et al.* 2000), achondroplasia, thanatophoric dysplasia and osteogenesis imperfecta (Orioli *et al.* 1995). The authors postulate that this may be due to germ-line mosaicism in older parents or that the genes involved may be localised to an area of unstable DNA.

1.4 Perinatal outcomes after in-vitro fertilisation – an overview of the literature

In the UK, an MRC working party was set up to look at the perinatal outcome of children conceived after IVF (MRC 1990). There was no control group, but figures were compared with national statistics. They reported that 23% of the births were multiple, compared with 1% in the UK general population. There was an increase in preterm birth, low birth weight and perinatal mortality. The main determinant of these outcomes was multiple births, with the gestational age and birth weight declining with the number of embryos replaced.

1.4.1 Number of embryos replaced

The Israeli IVF registry reported the outcome of IVF pregnancies between 1982-1989 and findings were similar to the MRC working party findings (Friedler *et al.* 1992). There was an increase in preterm labour, low birth weight and perinatal mortality, but again this was found mainly to be due to the high rate of multiple pregnancies. The recommendations from the authors were that the number of

embryos transferred should be reduced in order to reduce the multiple pregnancy rates.

Early studies of in-vitro fertilization implied that there was a relation between the number of embryos transferred and pregnancy rate (Kerin *et al.* 1983; Trounson *et al.* 1981) but it was also evident that there was an increase in multiple pregnancies with an increase in embryo transfer. Kerin et al suggested that embryo transfer should be limited to 2 and that the improvement in embryo quality and the success of embryo cryopreservation would lead to increases in successful pregnancies, but limit the number of multiple births and the related risks. Hershlag et al analysed factors associated with multiple births in IVF cases (Hershlag *et al.* 1990). The only difference between the multiple birth and singleton cases in terms of diagnosis, stimulation protocol, and fertilization processes was the number of embryos transferred.

In recent years it has become increasingly clear that this is indeed the case. Both the UK Royal College of Obstetricians and the Human Fertilisation and Embryology Authority (HFEA) have recommended two-embryo replacements in all women less than 40 years. Several authors are now advocating the replacement of only one embryo at a time (Gerris and Van Royen 2000; Hazekamp *et al.* 2000; Templeton 2000). Templeton states that the welfare of the IVF child is the most important issue. This advocacy cannot be addressed if outcome after IVF is primarily measured by pregnancy success rate, and those pregnancies are multiple, resulting in increased incidence of prematurity and associated morbidity and mortality. Templeton also indicated that there is evidence that serial one-embryo transfers may enhance cumulative pregnancy rates. "If a uterus is receptive, then all viable embryos may implant, but if the uterus is not receptive, none will implant, no matter how many are replaced. Putting back fewer embryos more often gives the uterus more opportunities to be receptive" (Templeton 2000).

IVF children are at an increased risk of preterm deliveries. In a study by Stromberg, 30.3% of all IVF study children were born before 37 weeks gestation and 6.7% before 32 weeks compared with 11.3% and 2.6% for IVF singletons which implied

that implantation of one embryo might reduce the prematurity rate by 60% (Stromberg *et al.* 2002).

Kerin's predictions are becoming reality and the improving success rate of frozen embryo transfer along with the development of criteria to assess embryo quality now means that single embryo transfer does not equate to increased numbers of IVF cycles. New studies assessing single embryo transfer are demonstrating the benefits and successful outcome of this approach (Gerris and Van Royen 2000; Vilska *et al.* 1999).

1.4.2 Perinatal outcome studies

(see table 1.1)

It is unsurprising that there is a greater increase in multiple pregnancies after IVF, but it would appear that singleton IVF pregnancies also carry greater antenatal risks than matched naturally conceived pregnancies. The incidence of preterm births and lower birthweight is increased if singletons and twins are considered separately (MRC 1990; Petersen *et al.* 1995; Gissler *et al.* 1995; Leslie *et al.* 1998; Dhont *et al.* 1999; Wennerholm and Bergh 2000; Koivurova *et al.* 2002).

The difficulty in establishing whether or not IVF singletons are at increased risk for poorer perinatal outcomes is that there are so many potential confounding factors, such as parental age, social class, maternal parity and underlying causes for infertility. The sociodemographic profiles and the parental ages are sometimes considered, but the underlying causes of infertility are numerous and may influence outcome. The ovarian stimulation drugs may themselves be implicated

A retrospective French study of 162 IVF singleton pregnancies, 263 pregnancies after stimulated cycles (without IVF) and 5096 naturally conceived pregnancies found no difference in complications between the IVF and stimulated cycle groups with respect to prematurity, low birth weight and perinatal mortality (Olivennes *et al.* 1993). However, both these groups had a worse outcome than the naturally conceived pregnancies, suggesting that the poorer outcome related to underlying

infertility or ovarian stimulation and to population characteristics such as maternal age, rather than the IVF process itself.
The large naturally conceived control group in this study, although more likely to be primigravid and younger, had a constant prematurity rate with increasing parity and age. Restricting the study to primigravidae mothers did not alter the outcome for caesarean section, prematurity, low birthweight or small for gestational age babies.

A large population based study of low birth weight babies in the United States, compared 42,463 infants conceived after assisted conception in 1996-7 with 3,389,098 infants born in the USA in 1997 (Schieve *et al.* 2002). Singleton, term, assisted conception babies had a 2.6 times increased rate of low birth weight. The authors suggest a possible causal factor may be the use of human menopausal gonadotrophin, which is associated with increases in insulin-like growth factor-binding protein 1 and this is linked to IUGR. Maternal hypertension and early elective caesarean sections are also likely to influence outcome.

A Finnish study aimed to investigate perinatal differences in children born to 107 women with unexplained infertility compared with two control groups from the Finnish medical birth registry (Isaksson *et al.* 2002). The first control group included spontaneous pregnancies and the second included all other pregnancies after IVF, ICSI or frozen embryo transfer (FET). Groups were matched for age, parity, number of children at birth and area of residence. There was no difference in risk between women with unexplained infertility and the control group of all pregnancies after IVF, ICSI and FET. No difference was found between any group for incidence of low birthweight (<2500g) or for mean birthweight among singletons and the authors argue that this reflects the high degree of matching between their groups compared to other studies.

Gissler et al also used the Finnish Medical Birth Register to study perinatal mortality and infants that were born with a birth weight <2500g (Gissler and Hemminki 1996). A regression analysis was performed taking into account sociodemographic factors such as smoking, age, marital status, gravida and parity and then the impact of IVF was assessed. The low birth weight and perinatal mortality was higher in the IVF

populations for singles and multiples. In this study, the mother's background did not explain the increased risks. When the results were analysed in terms of gestational length and plurality, differences between IVF and non-IVF children were reduced.

The authors argue high multiple and premature births are an inherent part of IVF and therefore matching for gestational length and multiples gives misleading results with respect to the total impact of IVF and provides an unrealistic view of total health risks and the cost of IVF treatments.

An increased rate of caesarian section (Dhont *et al.* 1999) and placenta praevia has been demonstrated to be more common in IVF groups (Tan *et al.* 1992; Verlaenen *et al.* 1995). Some studies have also found an increase in hypertension and vaginal bleeding (MRC 1990;Tan *et al.* 1992)

An epidemiological study in 1992 (Doyle *et al.* 1992) suggested that, contrary to previous hypotheses, increased maternal age and lower previous parity did not appear to be a risk factor for prematurity, low birth weight or SGA. However, hypertension during pregnancy was an independent risk factor in IVF pregnancies for preterm delivery, low birth weight and SGA. Bleeding during pregnancy was an independent risk factor for preterm delivery and the number of embryos and type of infertility an independent risk factor for low birth weight.

An increase in perinatal mortality of IVF babies has been suggested in some studies (Tan *et al.* 1992; Koivurova *et al.* 2002), but the first case-control analysis of contributing factors was reported by Draper (Draper *et al.* 1999). This study found that a history of infertility increased perinatal deaths irrespective of treatment. Untreated women with a history of infertility had a higher incidence of perinatal death when compared to women without infertility and this was unrelated to multiple births. The treated infertile women also had an increase in perinatal mortality that was only partly associated with multiple births. They concluded that women who experienced infertility had double the risk of perinatal death in the study period.

A Finnish birth registry study of IVF children compared with the general population also found that neonatal mortality was twice as high in the IVF population than the national figures (Koivurova *et al.* 2002). They conclude that although the statistical power of this study is very weak, there was evidence that the mortality rates among IVF children are mainly due to an increase in multiple births and the characteristics of infertile women, such as age.

As IVF children are more likely to be born preterm, whether or not they are part of a multiple pregnancy, they are more likely to require neonatal care (Leslie *et al.* 1992; Leslie *et al.* 1998; Koivurova *et al.* 2002; Gissler *et al.* 1995). The neonatal hospitalisation is prolonged when compared to controls (Tallo *et al.* 1995) and there is a higher incidence of mechanical ventilation (Leslie *et al.* 1992). The perinatal outcome of ICSI children has been found to be no different than conventional IVF with all the above factors applying (Wisanto *et al.* 1995).

Table 1.1 Perinatal outcomes after IVF

Authors	Study group	Study type	Outcome	Key results	Comments
Pinborg et al 2003	472 ICSI/IVF twins, 634 ICSI/IVF singletons, 1132 non-ICSI/IVF twins in Denmark in 1997.	National cohort study of twin and singleton births in Denmark	Questionnaire data for general health of children completed by mothers	Physical health of ICSI/IVF and non-ICSI/IVF twins were comparable. ICSI/IVF twins were more likely than ICSI/IVF singletons to be admitted to neonatal unit, require surgery and have poorer health as reported by mothers.	Parental questionnaire data-no objective medical data. Lack of information about non-respondents.
Schieve et al (2002)	424653 infants born in USA after assisted reproduction (1996-7) and 3,389,098 infants born in USA (1997)	Population based case control study. Subgroups matched for age and parity	Infant birth weight Low birth weight (LBW) <1500g Very low birth weight (VLBW) <2500g	Singleton term IVF infants at 2.6 times risk of LBW (95% CI 2.4-2.7). No increased risk LBW in twins. 0.6% births to mothers >20years, but include 3.5% LBW and 4.3% VLBW	Large study with good statistical power. Unable to conclude if outcome due to IVF per se or characteristics of infertile mothers.
Isaksson et al (2002)	90 IVF children from 107 Finnish mothers with unexplained infertility. 2 control groups from Finnish medical birth register (FMBR); group 1 - spontaneous pregnancy 445 women and 545 children; group 2 - all pregnancies after IVF	Case-control study. Group 1 matched for maternal age, parity, year of birth, number of children at birth, mothers residence.	Infant birth weight, gestation, perinatal mortality and multiple births	No difference in mean birth weight for singletons. No difference in gestation or perinatal mortality. Multiple pregnancy rate 23.3% in study group. More breech deliveries in study group (10.1%) $p<0.01$. Rate of asphyxia (diagnosed by paediatrician) higher in singletons in study group compared with control 1 ($p<0.05$)	Small study group. Close matching, but not for race.
Koivurova et al (2002)	304 Finnish IVF children and 569 from FMBR. 107 IVF singletons and 103 IVF twins compared with 287 singletons and 103 twins from FMBR.	Population based case-control. Matched for sex, year of birth, area of residence, parity, maternal age and social class.	Mortality rates, birth weight, prematurity	Compared with general population control group, IVF children at increased risk of prematurity-OR 5.6 (CI 3.7-8.6), neonatal morbidity-OR 2.4 (CI 1.7-3.4), VLBW-OR 6.2 (CI 2.0-19.0), LBW-OR 9.8 (CI 5.6-17.3)	Power too small to comment on mortality rates. Control group well matched for maternal age. Findings mainly due to large proportion of multiple births

Table 1.1 Perinatal outcomes after IVF (cont'd)

Authors	Study group	Study type	Outcome	Key results	Comments
Klemetti et al (2002)	IVF and other children from Finnish medical birth register 1991-3 and 1998-9	Retrospective population controlled study.	Multiplicity and use of health care services	Multiple birth rates, prematurity and low birth weight decreased from 1st to 2nd time frame. Prematurity, perinatal mortality and hospital admission>7days was increased in IVF groups compared to population	Questions for birth registry changed between 2 time frames. Missing cases cannot be quantified.
Draper et al (1999)	567 perinatal deaths in Leicestershire associated with 542 women 1990-4. Compared with 983 infants born to 972 women	Population based case-control study of perinatal deaths. Controls selected using proxy birth data form previous year which defined which births to include as controls.	Perinatal mortality rates.	Infertility increased risk of perinatal death-OR 6.2% (CI1.8-4.5). Compared to women without infertility, untreated and treated infertility increased perinatal risk (OR 2.9 (CI1.8-4.5). Mean gestation (SD) in weeks at delivery for cases 31.9 (6.1) and controls 39.2 (1.9)	Increased risk related to multiple births and gestation. Perinatal deaths in untreated infertility group were mainly singletons, whereas in the treated group they were mainly multiple births.
Leslie et al (1998)	95 Australian IVF infants and 79 naturally conceived controls	Case-control study. Matched for maternal parity and age.	Use of neonatal and health care resources in first year.	Singleton IVF neonates more likely to need NNU care) OR 3.04 (CI0.97-9.52) and duration of stay was longer (p=0.015). No over-use of health care after discharge in first year.	Small numbers. Considered multiple births separately. No increase in prematurity or low birth weight, but enrolled after 30 weeks.
Gissler et al (1996)	Comparison of 1335 IVF and 194,383 non-IVF children from Finnish medical birth registry data 1991-1993	Population controlled study.	Birthweight and perinatal mortality.	Low birth weight 30.6% in IVF and 3.9% in non-IVF groups (OR 7.94). Perinatal mortality 29.3% IVF and 7.3% non-IVF (OR 4.17%). OR reduces to 1.16 for LBW and 0.93 for perinatal mortality after adjusting for gestation and plurality.	Demonstrates the improved outcome results if adjust for gestation and plurality. Authors argue that prematurity and multiplicity are inherent part of IVF and adjustments are misleading.

Table 1.1 Perinatal outcomes after IVF (cont'd)

Authors	Study group	Study type	Outcome	Key results	Comments
Olivennes et al (1993)	162 singleton IVF babies, 263 babies conceived after stimulated cycle (non-IVF) and 5096 naturally conceived babies.	Case-control study	Prematurity, birth weight, perinatal mortality	Outcomes for IVF and non-IVF stimulated cycles similar for prematurity, birth weight and perinatal mortality. Both groups have poorer outcomes than naturally conceived group	Authors suggest that the differences in outcome are related to population characteristics of the subfertile groups or ovarian stimulation rather than IVF.
Tan et al (1992)	961 babies conceived after IVF at two fertility clinics. Control group of naturally conceived births obtained from two London hospitals.	Case-control study.	Obstetric outcomes of bleeding, hypertension, placenta praevia, prematurity and birth weight	IVF pregnancies resulted in an increase in bleeding, hypertension and caesarean section. IVF singletons pregnancies had an increased risk of IUGR, placenta praevia and prematurity.	Groups were matched for maternal age and multiplicity. Sociodemographic differences between the two groups were not considered
Doyle et al (1992)	Medical Research Council IVF register in UK 1978-1987. Study included 648 singleton liveborns.	Case group survey	Prematurity, birthweight.	Of IVF singletons: 13% preterm, 11% low birth weight and 17% small for gestational age. Multiple regression analysis showed hypertension to be a risk factor for all 3 outcomes, bleeding for prematurity and number of embryos and type of infertility for low birthweight.	No control group and retrospective study. Analysis of data for singleton IVF babies eliminates a large potential confounding variable when studying the effects of IVF per se.

1.5 Developmental outcome studies of IVF children.

1.5.1 Conventional IVF

(see table 1.2)

A Swedish study in 1996 assessed the cognitive, behavioural and social development of 99 children born after IVF (Cederblad *et al.* 1996). The study looked at IVF conceived children over an age range of 33-85 months and compared these to Swedish and American population norms, rather than a matched control group. As with many studies the IVF study group included a high rate of multiple births and prematurity. The researchers concluded that the results were "very satisfactory" with a Griffiths developmental quotient (DQ) above the Swedish norm. The authors did point out that this "norm" was due to be modified and comparing this recently evaluated IVF group with "old" normal values may not be representative of differences between IVF and non-IVF children.

Development of IVF children has been assessed by other researchers (Morin *et al.* 1989; Mushin *et al.* 1986; Yovich *et al.* 1986), but only with small numbers and few studies used naturally conceived controls (Morin *et al.* 1989). No increase in malformation rate was found. Brandes et al studied physical and mental development of IVF children and matched naturally conceived controls (Brandes *et al.* 1992). They found no statistically significant difference in developmental indices. As with other studies there was a higher rate of preterm, multiple births and low birth weight in the IVF group of children. Children from multiparous pregnancies had generally lower developmental and physical scores.

Table 1.2 Developmental outcome studies for conventional IVF children

Authors	Study group	Study type	Outcome	Key results	Comments
D'Souza et al (1997)	278 IVF and 278 naturally conceived UK children. IVF singletons mean 25.5 months. IVF multiple births mean 24.8 months	Prospective case-control study. Matched for sex and social class.	Results of Griffiths scales of development	Mean developmental quotient (DQ): IVF singletons 116.9 (SD 12.6). IVF multiple births 106.9 (SD 10.9). Not stated for controls*. Developmental delay (DQ<70) noted in 2 multiple birth IVF children only.	46% IVF children from multiple births. All controls were singleton. No matching for prematurity, birth weight or gestation.
Cederblad et al (1996)	99 Swedish IVF children (age 33-85 months)	Single cohort compared to Swedish and American norms	Results of Griffiths scales of development	Developmental quotient (DQ) above Swedish norm	No matched control group. High numbers of multiple births and prematurity
Brandes et al (1992)	116 Israeli (Hebrew speaking) IVF children and 116 matched non-IVF children (age 12-45 months)	Case-control study. Matched for birth weight, gestational age, birth order, order in multiple births, mode of delivery, sex, age, maternal age & education.	Bayley scales for infants up to 30 months. Stanford-Binet scales for children>30 months. Scales mean 100 ±16	MDI Bayley scores: IVF 106 ±19.6 Non-IVF 110.6 ±19.3 Composite Index for Stanford-Binet IVF 106.2 ±8 Non-IVF 104.4 ±10.2	No correction for prematurity because children all>12 months.
Morin et al (1989)	83 IVF children from Norfolk, USA and 93 matched non-IVF children (age 12-30 months)	Case-control study. Matched for age, sex, race, multiple births and maternal age.	Results of Bayley scales: mean developmental index (MDI) and physical developmental index (PDI). Mean score 100	MDI scores: IVF 115 ±13 Non-IVF 111 ±13 PDI scores: IVF 114 ± 14 Non-IVF 108 ±15	Study had power of 99% to detect difference. Strongly suggests no difference. However, scores corrected for prematurity.
Mushin et al (1986)	33 Australian IVF children (age 12-37 months)	Single cohort from first 52 infants conceived at Monash IVF centre. No matched controls.	Results of Bayley scales. One child (37 months assessed using McCarthy scales	Overall MDI of 111 (SD=15) and PDI of 105 (SD=23). 4 children with physical and developmental problems had lower scores.	High numbers of multiple births and prematurity. Of 4 children with poor scores: 2 VLBW, 1 severe CHD.
Yovich et al (1986)	20 Australian IVF children (age 12-13 months)	Single cohort of first 20 infants conceived after IVF in Western Australia	Results of Griffiths scales of development	General developmental quotient (GQ) was greater than mean of 100 in 19/20 children after correction for gestational age.	No matched control group. Increased rate of multiple births, IUGR, prematurity and caesarean section.

*Quoted in previous paper: D'Souza et al Clinical IVF forum: current views in assisted reproduction 1990:70-78 (D'Souza et al. 1990)

1.5.2 Intracytoplasmic Sperm Injection (ICSI)

(see table 1.3)

The first paper to report developmental differences in an ICSI group of children when compared to conventional IVF and naturally conceived controls was published in 1998 (Bowen *et al.* 1998). They found an increase in mild developmental delay using the Bayley Scales of Infant Development to derive a mental development index. However, the study used comparison groups of IVF and naturally conceived children that were already enrolled in a separate study and had differing demographics to the ICSI group. There was also no blinding of the assessors and the number of participants in the study was small with 89 ICSI conceived children.

This same cohort of children has recently been studied at age 5 years (Leslie *et al.* 2003). 85% of the original cohort participated in this follow up study and additional children were recruited to increase the power of the study, although the total numbers remain small, with 97 ICSI children, 80 IVF and 110 naturally conceived controls. These children were assessed using the Weschler Preschool and Primary Scales of Infant Intelligence (WPPSI). No differences were found between children conceived after ICSI, IVF or naturally for mean full IQ scores. The only independent predictor of below average IQ was maternal educational level.

Bonduelle et al have published several papers investigating congenital malformation rates and physical development of ICSI children (Bonduelle *et al.* 1996b; Bonduelle *et al.* 1996a; Bonduelle *et al.* 1998b; Bonduelle *et al.* 1998c; Bonduelle *et al.* 1999; Bonduelle *et al.* 2002). Several of these papers allude to the fact that developmental milestones were assessed using the Bayley scales, but the initial results of formal assessments of these children, undertaken between 1995-1998, were published in a research letter to the Lancet in 1998 (Bonduelle *et al.* 1998b). This article reported 201 ICSI children and 131 IVF children who were assessed using Bayley scales and the results were compared to a subset of children representing the Dutch population. The age of the children was not corrected for gestational age, but the ICSI and IVF children were found to have similar scores to the general population. The twins scored slightly lower than the singletons.

More recently, Bonduelle's group have published a prospective study of 439 ICSI children and 207 IVF children (Bonduelle *et al.* 2003).Children were assessed at 24-28 months by a paediatrician blinded to mode of conception and using the Bayley scales. There were no developmental differences noted between groups. Increased parity, sex (male) and younger age were the only variables to negatively affect the test results. However, this study lacked a naturally conceived control group and no conclusions can be made about development in comparison to the general population.

Sutcliffe et al studied 208 singleton ICSI conceived children at around 18 months and compared them with a matched naturally conceived control group (Sutcliffe *et al.* 1999; Sutcliffe *et al.* 2001). The children were assessed by a single observer using the Griffiths scales of mental development. No differences in developmental outcome were found between the two groups. These children were also included in a study comparing this UK cohort with a smaller cohort in Australia. No differences in neurodevelopment were seen between groups or countries (Sutcliffe *et al.* 2003).

Table 1.3 Developmental outcome studies for ICSI children

Authors	Study group	Study type	Outcome	Key results	Comments
Sutcliffe et al (2003)	Australia: 58 ICSI, 38 naturally conceived (NC). UK: 208 ICSI, 221 NC	Retrospective case-control	Results of Griffiths scales of infant development	ICSI Australian 103.7(SD13.1) NC Australian 103.6 (SD 11.1) ICSI UK 98.08 (SD 10.93) NC UK 98.69 (SD 9.99)	Non-blinded. Participation 90% in UK, 85% in Australia. Scales not standardised in Australia.
Bonduelle et al (2003)	439 Belgium children conceived after ICSI compared with 207 children conceived after IVF. Age 24-28 months	Blinded prospective case-control study	Results of Bayley scales of Infant development	MDI Bayley scores: ICSI singleton 153.6 (SD 6.07) IVF singleton 154.3 (SD 5.75) ICSI twin 149.2 (SD 9.27) IVF twin 149.6 (SD 9.45)	No naturally conceived group. All scores higher than population mean. 12.2% total ICSI children and 11.9% total IVF children were assessed. Sperm parameters of fathers did not affect outcome.
Leslie et al (2002)	97 ICSI, 80 IVF and 110 naturally conceived children.	Case-control study. In part a follow-up from Bowen et al 1998 (but also new recruits)	Weschler Preschool and Primary Scales of Intelligence.	Full scale IQ: ICSI 110 (+/-18) IVF 111 (+/- 13) Non-IVF 114 (+/-13)	Multivariate analysis – only predictor of low score was maternal educational level. Findings differ from original study at one year (Bowen 1998)
Sutcliffe et al (2001)	208 UK ICSI children compared with 221 naturally conceived controls. Age 12-24 months.	Case-control study. Matched for social class, maternal educational level, region, sex and race.	Results of Griffiths scales of infant development.	Griffiths quotients: ICSI 98.08 (SD10.93) Controls 98.69 (SD 9.99)	No correction for gestational age in Griffiths scales. Single observer. 90% follow-up.
Bowen et al (1998)	89 Australian ICSI children compared with 84 conventional IVF children and 80 naturally conceived. Assessed at birth and at corrected age of 12 months.	Prospective case-control study. Matched for parental age, parity and multiplicity of the pregnancy. Conventional and IVF children were recruited through separate study.	Results of Bayley scales of Infant development	98% follow up at one year. MDI Bayley scores: ICSI 95.9 (SD 10.7) IVF 101.8 (SD 8.5) Non-IVF 102.5 (SD 7.6)	Included frozen embryos (39% ICSI, 31% IVF). Lack of blinding and differences in sociodemographic factors, particularly between the parents of the ICSI group and other groups.

1.6 Physical Assessments

1.6.1 Use of medical services

IVF children are more likely to need neonatal care, primarily because of the prematurity related to multiple pregnancies but, as previously discussed, there is also an increased risk of prematurity and low birth weight in singletons.

However, some studies suggest that IVF children appear not to require extra medical attention after the neonatal period (Leslie *et al.* 1998; Saunders *et al.* 1996). Leslie studied 95 IVF children and compared them with 79 naturally conceived children matched for maternal age and parity. Neonatal outcomes were similar in each group, although the IVF singletons had a significantly longer stay in NICU than controls. IVF children were also less likely to be breastfed by the time of discharge.

The utilisation of health services was similar between the two groups. Of those that over-utilised services, the IVF children were more likely to see health visitors and the control group were more likely to attend their GP. One fifth of the IVF children were admitted to hospital in their first year and over 50% of these admissions were surgical, mainly correction of congenital malformations.

An early study in 1989 included 83 IVF children with 93 matched controls and a full physical examination was performed including echocardiography, ECG, abdominal USS and cranial USS (Morin *et al.* 1989). No differences were found.

1.6.2 Growth

Saunders et al published a case-matched control study of children conceived after assisted reproduction and found that the physical outcomes: weight, head circumference and malformation rates were no different between groups (Saunders *et al.* 1996). The IVF group had a greater mean length percentile and the twins in each group had poorer physical outcomes with an increase in prematurity and lower birth weights, and reduced height and weight at age two when compared to singletons in each group.

1.6.3 Ocular abnormalities

The increase in multiple births and premature births related to assisted conception has led to an increase in conditions such as retinopathy that are directly related to preterm birth and low birth weight.

A study in Leeds found that 20% of babies born after assisted conception fulfilled ROP screening criteria and 25% of babies reaching threshold disease requiring treatment were assisted conception babies (McKibbin and Dabbs 1996). A more recent study in London reported similar findings with assisted conception babies accounting for 29% of babies requiring ROP treatment (Watts and Adams 2000). These studies were small and retrospective and there were no specific matched control groups.

Anteby et al reported the ocular manifestations in children born after IVF and referred for opthalmological assessment. Major ocular malformations were found in 12 (26%) of the small cohort of 47 children studied (Anteby *et al.* 2001). Seven major malformations were listed, including congenital cataract, optic atrophy and retinoblastoma. The study was limited in power due to the small numbers of children involved and, because the study was conducted in a tertiary hospital, it is possible that the numbers were skewed due to the type of patients referred.

However, this report by Anteby et al was the first to note retinoblastoma in an IVF conceived child. Cruysberg and colleagues subsequently reported a case of bilateral retinoblastoma in a child conceived after IVF (Cruysberg *et al.* 2002) and a Dutch report of retinoblastoma in five children conceived after IVF suggest that this phenomenon needs further investigation (Moll *et al.* 2003). Moll suggests that the relative risk of developing retinoblastoma after IVF could be 4.9-7.2. This is calculated on the assumption that 1.0-1.5% of children born in the Netherlands in 2000-2002 were conceived after IVF. It is possible that the incidence of IVF was higher than this and that the relative risk is therefore smaller than reported.

1.6.4 Childhood Cancer

In addition to reports of an increase in retinoblastoma, there have been concerns raised about the possible increased risk of other cancers in IVF children. There have

been case reports of children conceived after assisted conception developing neuroectodermal tumours (White *et al.* 1990; Kobayashi *et al.* 1991) but no large study has confirmed this finding. Bruinsma et al used a record-linkage cohort design to link assisted reproduction births to a population-based cancer registry in Australia (Bruinsma *et al.* 2000). This study included 5249 births and found no increase in the incidence of cancers in the assisted reproduction groups. However, these groups were relatively small and underpowered for the outcomes measured. The mean length of follow up was only 3 years 9 months, although neuroblastomas tend to occur within the first year of life. These findings were supported by a smaller, similar Israeli study (Lerner-Geva *et al.* 2000).

More recently, Klip et al examined a large population-based historical cohort, established to investigate gynaecological disorders in women undergoing IVF (Klip *et al.* 2001). This cohort included 9484 children whose mothers had been given IVF or related fertility treatments and 7532 children whose mothers were subfertile, but had conceived naturally. The mothers were mailed questionnaires enquiring about cancer in their children. There was a 67% response rate and no difference between the groups was noted, implying that IVF and related treatments do not increase the cancer risk to the child.

The cancer incidence in IVF children studied for the UK MRC working party (Doyle *et al.* 1998) and a Swedish national cohort study of IVF children (Bergh *et al.* 1999) also found no increase in cancer rates, but the power of these studies was limited by the small number of children studied. Doyle estimated that 20,000 children would be required to observe a doubling or halving of the risk of childhood cancer in children conceived after assisted reproduction compared with the general population. This would provide 95% significance and 90% power if children were followed up for five years (Doyle *et al.* 1998).

1.6.5 Neurological outcomes

There has been some suggestion from a Swedish study that children born after IVF have an increased risk of developing neurological problems, particularly cerebral palsy (Stromberg *et al.* 2002). They found a four-fold increase in risk of cerebral palsy in children born after IVF compared with matched controls - OR 3.7 (95% CI

2.0-6.6). The risk in singletons was nearly three times – OR 2.8 (95% CI 1.3-5.8). After adjusting for birthweight and a gestation of >37 weeks, the risk remained with an OR of 2.5 (95% CI 1.1-5.2). The authors admitted that the frequency of cerebral palsy in controls was lower than the Swedish norm. Calculations using their data indicate a prevalence of cerebral palsy in the control group as 1.5/1000 compared with an accepted prevalence rate of 2.0-2.5/1000 (Healy and Saunders 2002). The increased risk was shown to be mainly associated with multiple births, low birthweight and low gestational age, but an effect of IVF per se could not be excluded.

This type of study has many possible confounding variables – not all can be examined in the analysis. Leviton and colleagues noted that there was some over aggregation of the data, with children less than 30 weeks gestation grouped together. This did not allow for the effect of decreasing risk of cerebral palsy with increasing gestation, particularly in those infants born after 30 weeks (Leviton *et al.* 2002).

In addition, there was no analysis of the chorionicity or zygosity of twins in the IVF and control groups (Davies and Norman 2002; Akande and Murphy 2002). Monochorionic and monozygotic twins are at increased risk of perinatal complications and cerebral palsy (Pharoah 2002). As well as monozygotic twins, dizygotic twins born after IVF are at increased risk of lower gestation, lower birthweight and lower apgar scores (Davies and Norman 2002). These are all factors associated with cerebral palsy.

There is also the possibility of a survival effect. If the rate of intrauterine death and early neonatal death differed between the IVF and control groups, the effect on the survivors might differ. Death of a co-twin is associated with an increased risk of cerebral palsy (Akande and Murphy 2002).

1.7 Congenital anomalies

In the UK, congenital anomalies are the most common cause of death in children aged between four and 52 weeks of life. In 1998, they accounted for 24% of deaths in this age group (UK Office of National Statistics). Birth defects of sufficient magnitude to require medical intervention occur in 3-5% of newborns (Genetic Drift

1995). The causes of most birth defects remain unknown, but there is much research into genetic risk factors.

Genetic variants may be susceptible to many environmental factors, which may result in birth defects. About 5% of congenital anomalies are thought to be related to maternal exposures to "environmental" agents, e.g. drugs, viruses, maternal metabolic disorders, or other exposures that may occur prenatally. Table 1.4 outlines the aetiology of malformations in newborn infants.

Table 1.4: Aetiology of Congenital Malformations in Humans

Causes of congenital malformations	**% of all defects**
Mendelian disorders – Single gene eg achondroplasia; cystic fibrosis	20%
Chromosomal disorders **eg trisomies (13, 18, 21); Turner syndrome**	**10%**
Environmental causes - Teratogens Infections 1% Maternal disorders 1-2% Therapeutic radiation <1% Drugs and Chemicals 2%	5%
Unknown cause and multifactorial malformations	**65%**

Taken from Teratogen Update Vol 12 (Genetic Drift 1995).

Concerns have been raised that the process of in-vitro fertilisation, particularly after ICSI may lead to an increase in birth defects. If this is the case, there does not appear to be any pattern that suggests that the process might be caused by a single aetiological factor.

If the assisted reproduction process exposed the sperm, oocytes or embryos to a classical teratogen it would be expected that this would result in a recognisable pattern of birth defects because classical teratogens interact with a particular and

consistent cellular receptor. However, other mutagens are indiscriminate in the types of cells they may damage and therefore a less predictable pattern of malformations is seen. Despite this, many studies have shown that the most susceptible organ to mutagenic damage is the nervous system, particularly the brain, and that microcephaly is the most consistent finding in non-lethal mutagenic damage. There are some published studies of IVF and ICSI children, which suggest an increase in neural tube defects (Ericson and Kallen 2001), but the majority of studies have not found any increase in specific organ malformations.

Sperm DNA in subfertile men has been shown to have increased levels of strand breakages which may be worsened during exposure to chemicals during the IVF process (Cummins and Jequier 1995). These breakages could potentially be passed to children, with deleterious effects. Most evidence so far suggests that if there is any increase in malformations following ICSI, it is more likely to be due to abnormal sperm parameters rather than a mutagenic effect from exposures during the assisted reproduction process (see section 1.8).

A comparative study of IVF/ICSI and naturally conceived children may indicate a higher rate of subtle malformations or genetic abnormalities, but only large studies comparing ICSI with conventional IVF can differentiate the effects of oligoasthenoteratozoospermia from the effect of the culture media used and exposures that occur during the assisted reproduction processes.

1.7.1 Studies investigating the incidence of congenital malformations in IVF

Many studies have reported rates of congenital malformations in IVF children (see table 1.5). However, the results of these studies are extremely difficult to compare and require careful interpretation. Most birth defect registries are likely to under-report, particularly minor malformations. Studies investigating children conceived after assisted reproductive technologies may report higher incidences of malformations because researchers and clinicians may look more closely at this group. The definitions of major malformations and the classification methods used also differ between study groups. In addition, the numbers of children involved in these studies, in almost all cases, is too small to provide a statistically meaningful rate of anomaly.

Many of these studies have not had adequate matched control groups and there are variations in the classifications of major and minor anomalies. Simpson suggested that major anomalies should be defined as defects resulting in death, major handicap or necessitating surgery (Simpson 1996). He suggested that surveillance for anomalies should be performed prospectively from the time of diagnosis of pregnancy. Internal anomalies should be recorded separately as these would be difficult to compare with the naturally conceived population as anomalies not obvious at birth are seldom, if ever, included in congenital malformation studies or registers. He also warned against pooling anomalies in liveborns and abortuses because this data cannot be compared to birth defect registries for the general population, which do not record anomalies in abortuses.

Table 1.5 Congenital malformation studies for IVF children

Authors	Study group	Study type	Outcome	Key results	Comments
Anthony et al (2002)	4224 IVF children and 314605 naturally conceived children compared using records from Dutch national database 1995-1996	Population based case-control study	Congenital malformation rates	Congenital malformations were found in 3.2% IVF children and 2.7% naturally conceived. OR for risk of malformation in the IVF group: 1.20 (95% CI 1.01-1.43. OR of cardiac malformations was higher in IVF group 1.56 (95% CI 1.10-2.22.	Largest study so far with records from the same database. Unable to differentiate ICSI and conventional IVF children. National database tends to under-report approx 17% of congenital malformations at birth.
Koivurova et al (2002)	304 Finnish IVF children compared 569 children from the general population and plurality matched control group. Randomly chosen from the Finnish Medical Birth Register.	Population-based cohort study	Incidence of malformations	An overall malformation rate (major and minor) of 6.6% was found in the IVF group and 4.4% in the general population control group. There was a 4-fold increase in the prevalence of cardiac anomalies in the IVF group compared with controls.	Singleton control group well matched for maternal age and parity. 304/306 IVF children conceived during 1990-1995 traced. Congenital malformations picked up after the neonatal period included.
Hansen et al (2002)	301 Australian ICSI conceived infants compared with 837 conventional IVF infants and 4000 naturally conceived infants	Case-control study	Incidence of malformations	Major malformations: (8.6%) ICSI infants; (9.0%) IVF infants (4.2%) naturally conceived	Classification of major defects differs from other standard definitions. Used 3 different registers. Groups not well matched.
Bonduelle et al (2002)	2889 ICSI children compared with 2995 conventional IVF children	Prospective cohort study	Congenital malformation rates	Major malformations observed at birth in 3.4% live born ICSI children and 3.8% IVF children.	Findings were recorded in the neonatal period. No details of malformations identified in the follow up period were given.
Sutcliffe et al (2001)	208 UK singleton ICSI-conceived children matched with 221 naturally conceived children	Case-control study	Rates of congenital abnormalities	Major congenital malformation rates: 10 study children (4.8%) and 10 (4.5%) control children.	Children of oligospermic fathers had higher proportion of genitourinary defects.
Foresta et al (2001)	Analysis of Sutcliffe data (above). Children of oligozoospermic men.	Case-control study	Congenital abnormalities	8/10 (80%) major anomalies and 17/22 (77.3%) minor anomalies found in children conceived to oligospermic men	Also screened 680 oligozoospermic men for genetic causes of infertility and found prevalence of 12.2%.
Ericson et al (2001)	Swedish IVF register from 1982-1997. Population-based control group	Population-based case-control study	Congenital malformation rates.	Odds ratio (OR) stratified for year of birth 1.47 (95% CI 1.34-1.61). OR reduced to 1.39 when maternal age and parity was taken into consideration. Stratification for singletons reduced OR to 1.24.	Increased risk of congenital malformations in IVF population appears to be an effect of the characteristics of the women undergoing IVF.

Table 1.5 Congenital malformation studies for IVF children (cont'd)

Authors	Study group	Study type	Outcome	Key results	Comments
Wennerholm et al (2000)	1139 Swedish infants born after ICSI. Incidence compared with Swedish birth registry and congenital malformation register	Population control case series	Incidence of congenital malformations.	Odds ratio (OR) for minor or major malformations 1.75 (95% CI 1.19-2.58) Hypospadias was only malformation found to occur in excess in ICSI children.	Medical birth registry not fully complete – may lead to exaggerated ICSI risk.
Westergaard etal (1999)	Danish National IVF Registry 1994 and 1995. 2245 IVF children and equivalent controls matched for maternal age, child age, parity and multiple births.	National case-control study	Malformations found at birth.	107 (4.8%) study children were born with malformations. 45.8% were born in multiple pregnancies. 103 (4.6%) control children had malformations of which 60.2% were from multiple pregnancies. The background malformation rate of population was 2.8%.	Included ICSI, IVF, frozen embryo replacement and egg replacement. Compulsory register. Multiplicity rather than assisted conception appears greater risk factor.
Bonduelle et al (1998)	1987 children conceived after ICSI.	Prospective cohort study	Congenital malformation rates over time.	2.3% major malformations at birth. 7 malformations interrupted, 21 (1.1%) stillbirths–4 with major malformations. Prenatal karyotypes for 1082 found 18 abnormal, de novo malformations (1.66%).	Chromosome malformations possibly due to characteristics of infertile men rather than ICSI technique.
Bonduelle et al (1995)	130 children conceived after ICSI compared with 130 conceived after conventional IVF	Prospective cohort study	Congenital malformation rate at birth and 2 months.	4 major malformations in ICSI group. 6 major malformations in IVF group.	Not compared with general population because of risk of looking more carefully in the study population.
Freidler et al (1992)	Israeli IVF register 1982-1989. 1475 children conceived after conventional IVF.	Case series	Congenital malformations at birth.	Major congenital malformation rate 2.2%	Comparable with general population.
Wennerholm et al (1991)	131 Swedish children conceived after conventional IVF.	Case series	Incidence of congenital malformations in infancy.	3/131 infants (2.3%) noted to have congenital malformations	Initial cohort of IVF children in Sweden. Similar incidence to general population.
Morin et al (1989)	83 IVF children in Norfolk,USA compared with 93 non-IVF children (aged 12-30 months)	Case-control study.	Incidence of major congenital malformations	Major malformations in 2/83 (2.4%) IVF children and 1/93 (1.1%) non-IVF children.	Non-significant differences. Small study. Matched for age, sex, race, multiple births and maternal age.

Table 1.5 Congenital malformation studies for IVF children (cont'd)

Authors	Study group	Study type	Outcome	Key results	Comments
Oliviennes et al (1997)	422 French children conceived after IVF aged 6-13 years. Data mainly collected using questionnaires.	Case series	Incidence of malformation	One child with Downs syndrome. No other major malformations. Total malformation rate 2.7% (9/370).	Classification of major and minor malformations not clear. Children were not examined
Saunders et al (1996)	314 Australian IVF children and 150 control children selected from perinatal data collection unit records. 1991-1993.	Case-matched control study	Incidence of malformation	The number of malformations in each group was similar. There was no increased rate of malformations after controlling for prematurity and gestation.	The methods of recruitment changed after 1 year. All participating children reviewed by paediatrician.
French National IVF registry (1995)	Pregnancies and births after IVF during 1986-1990. 6879 infants born after 5371 deliveries.	Prospective multicentre survey	Congenital malformation rates of births and stillbirths.	Malformation rate after IVF 2.8% compared with 2.1% in general population. Cardiomyopathy was most common major malformation and urogenital tract anomalies accounted for 20% of minor malformations	Prevalence of congenital malformations in general population obtained from EEC (Eurocat) and a Paris registry of births and abortions.
MRC Working Party (1990)	UK children conceived after IVF. Included 1267 pregnancies with 1581 liveborn or stillborn children. Included GIFT and IVF.	National survey (no controls)	Prevalence of congenital malformation.	Malformations diagnosed at any age were reported in 2.9% (46/1581) babies. This included 5 terminations for major malformations. 2.2% had one or more malformations detected in the first week of life.	Data collected using different methods in England, Wales and Scotland. Some methods use voluntary reporting – this may underestimate the total numbers. The possibility of more careful reporting of IVF children is also noted.

There has been one systematic review of this subject performed by a Norwegian team (Tanbo 2002). This study evaluated 13 cohort studies with adequate and well-defined control groups and 17 other case series or cohort studies with weak or non-defined control groups. A meta-analysis of birth defects included nine studies comparing conventional IVF and ICSI and found a risk of birth defects of 1.14 (95% CI 1.01-1.30), suggesting a small increased risk of major birth defects in children conceived after treatment of severe male infertility with ICSI.

The largest prospective cohort study of IVF and ICSI children is by Bonduelle et al in Belgium. The most recent report compared 2889 ICSI conceived children with 2995 IVF conceived children (Bonduelle *et al.* 2002). Major anomalies (defined as malformations requiring surgery or causing physical impairment) were observed at birth in 3.4% live born ICSI children and 3.8% IVF children. After including stillborns and terminations, this rose to 4.2% in the ICSI group and 4.6% in the IVF group. However, there was no naturally conceived control group and because this study compared ICSI and IVF children after very detailed neonatal examinations, it is hard to compare the data to the general population where the levels of reporting will differ.

The total anomaly rates (major and minor) using the Manual of the International Statistical Classification of Diseases, Injuries and causes of Death - ICD-10 (WHO) were 6.7% in the ICSI children and 9.0% in IVF children. This was largely attributed to ultrasound scan (USS) findings, mainly in IVF twins on the neonatal unit. When children who had 2 or more USS were excluded, the rate dropped to 4.4% ICSI and 5.3% IVF. Anomaly rate in ICSI children was not related to sperm origin or quality.

A report of this cohort in 1998 with 1,987 children conceived after ICSI (Bonduelle et al 1998) found no overall increase in malformation rate compared with national statistics for the general population. However, there was a significant increase in *de novo* chromosome aberrations and a higher frequency of transmission of sex chromosome aberrations. Prenatal karyotypes for 1082 found 18 abnormal and *de novo* malformations (1.66%); half of these were sex chromosomal abnormalities. Ten karyotypes showed inherited structural aberrations, considered to be due to the characteristics of subfertile fathers rather than the ICSI technique per se.

Several countries have published national data (i.e. UK, Israel, France, Australia and Denmark) and the congenital malformation rates appear to be similar to national population data (MRC 1990; Friedler *et al.* 1992; Olivennes *et al.* 1997; Saunders *et al.* 1996; Westergaard *et al.* 2000). However, the numbers of children included in these studies was relatively small and therefore statistical power was weak.

A report from the compulsory Danish IVF registry from 1994-1995 (Westergaard *et al.* 2000) looked at data for perinatal outcome, cytogenetic findings and fetal malformations of children conceived after conventional IVF, ICSI, frozen embryo replacement and egg donation. This data was compared with a control group from the Danish Medical Birth Registry matched for maternal age, parity and multiple births. There was no significant difference between the study groups for malformations, but both groups had higher malformation rates than the general population rates. One hundred and seven (4.8%) study children were born with malformations of which 45.8% were from multiple pregnancies. One hundred and three (4.6%) control children had malformations of which 60.2% were from multiple pregnancies. The malformation rate of the general population was 2.8%. The study concluded that it is multiple pregnancies and individual patient characteristics that determine malformations rather than the IVF process.

Data from the Swedish IVF registry (Ericson and Kallen 2001) also found that an increased risk of congenital malformations in IVF population appeared to be an effect of the characteristics of the women undergoing IVF. The odds ratio (OR) for congenital malformations in IVF compared with a population control was 1.47 (95% CI 1.34-1.61) when stratified for year of birth. This reduced when maternal age, parity and stratification for singletons was taken into consideration.

However, the authors found a specific increase in neural tube defects, alimentary tract atresia, omphalocoele and, in the ICSI group only, hypospadias. The authors hypothesised that the increase in neural tube defects, particularly spina bifida and anencephaly in the IVF population may be the reluctance of parents of the children to accept the offer of abortion in these cases, particularly if the affected child has a normal twin.

In an earlier Swedish study, neural tube defects and oesophageal atresias were found to be increased in IVF children (Bergh *et al.* 1999). Alimentary tract atresias are thought to be increased in monozygotic twins (Ericson and Kallen 2001)and monozygotic twinning is known to be increased in IVF (Abusheikha *et al.* 2000). It may therefore be the case that the increase in atresia is secondary to the monozygotic twin rate associated with IVF, rather than the IVF process itself.

An increase in cardiac malformations has been reported in some studies (Anthony *et al.* 2002; Koivurova *et al.* 2002). Anthony et al showed that all specific cardiovascular malformations were more frequently observed in IVF children with an odds ratio of cardiac malformations in the IVF group of 1.56 (95% CI 1.10-2.22) when compared with naturally conceived children. However, the occurrence of single umbilical artery was the only malformation to reach significance. Koivurova found a four fold increase in cardiac malformation compared to naturally conceived controls. Bonduelle and Koivurova also noted that there was a higher rate of cardiac malformation in multiple birth children (Bonduelle *et al.* 2002). It is likely that the presence of single umbilical artery, persistent ductus arteriosus and other similar cardiac abnormalities are more likely to be detected in premature infants admitted to the neonatal unit.

An increased rate of genitourinary anomalies in ICSI children has also been reported in case reports (Macnab and Zouves 1991) and in other studies (Sutcliffe *et al.* 2001; Wennerholm *et al.* 2000). Wennerholm studied the malformation rate in ICSI children and compared these results with the general population. The only malformation found to be specifically increased in ICSI children was hypospadias. The author also commented on a higher rate of congenital malformations in multiple birth ICSI children. Others have also suggested that there may be an increase in hypospadias in children conceived after ICSI (Anthony *et al.* 2002).

Sutcliffe et al found that genitourinary anomalies occurred mainly in the subgroup of ICSI children conceived to oligospermic men. Foresta and Ferlin performed more detailed analysis on Sutcliffe's data and hypothesised that genetic alteration causing infertility in fathers could lead to a genitourinary phenotype in their children (Foresta and Ferlin 2001).

Foresta and Ferlin's analysis of Sutcliffe et al's data also noted higher rates of congenital malformations per se in fathers with oligozoospermia;. 8/10 (80%) of the major anomalies and 17/22 (77.3%) of minor anomalies were found in children conceived to oligozoospermic men.

1.8 Specific concerns about Intracytoplasmic Sperm Injection

ICSI is now widely used to treat male infertility and in other conditions where conventional IVF treatment has not been successful. Concerns have been raised that infertility is being treated successfully in many cases where the underlying cause of the infertility is not known. It is therefore not possible to predict the outcome for the children in terms of their potential for infertility or genetic defects.

Embryologists select the sperm to be injected into the oocyte, bypassing the natural selection process of normal conception and also conventional IVF. Morphological criteria have been used to assess the suitability of sperm used in the fertilisation process. Viville et al have demonstrated that these criteria are unpredictable and that morphologically abnormal sperm may have normal chromosomal status (Viville *et al.* 2000).

1.8.1 Genetic transmission of infertility

Tiepolo and Zuffardi first described the association between a deletion on the distal portion of the long arm of the Y chromosome and azoospermia in 1976 (Tiepolo and Zuffardi 1976). It is now thought that 5-20 % of men with severe oligospermia or azoospermia are likely to have deletions in the long arm of the Y chromosome (Vogt *et al.* 1995). Kent-First et al studied 32 infertile men and their sons to investigate the incidence of Y linked microdeletions in the sons of infertile fathers conceived after ICSI (Kent-First *et al.* 1996). Three father/son pairs (9.4%) were found to have Y chromosome microdeletions. One father/son pair had the same microdeletion. The other two boys displayed the microdeletion, but their fathers did not.

The authors hypothesised that the two fathers whose blood sample did not demonstrate microdeletions could be mosaic for the deletion and that this was a de-novo deletion that may or may not be passed to their sons. However, if this was the case, then the father/son pair with the same microdeletion may have inherited it from

a de novo deletion in the baby's grandfather. The implication of this study is that the transmission of microdeletions to male children conceived after ICSI may lead to infertility in this second generation.

Page et al also looked at fathers and sons. They examined the Y chromosomes of 4 sons born to 3 fathers with Y chromosome deletions. The children were all found to have inherited the same Y chromosome deletion and are thus all likely to be naturally infertile themselves (Page *et al.* 1999).

1.8.2 Transmission of chromosomal abnormalities

Bonduelle at al 2002 found abnormal karyotypes in 2.9% of prenatally tested ICSI conceived fetuses (1.56% were de novo, 1.18% inherited). IVF conceived fetuses had abnormal karyotypes in 3.0% of cases, but this appeared to be related to maternal age. Approximately 5% of ICSI children were at increased risk of chromosomal aberrations due to parental chromosomal abnormalities (usually male), compared with 0.5% in the general population. Three ICSI children and three IVF children were born with clinically recognisable chromosome abnormalities (Bonduelle *et al.* 2002).

De novo sex chromo aberrations (Bonduelle *et al.* 1998c) have been identified in cases of men with severe oligoasthenoteratozoospermia and several researchers have also reported increases in sex chromosome abnormalities in ICSI children (In'tVeld *et al.* 1995; Liebaers *et al.* 1995) compared with the population rates. A review of nine studies suggested that the rate of chromosomal aneuploidy, nullisomy, disomy and diploidy appeared to be increased in the sperm of oligospermic men who were candidates for ICSI, but the rates varied from 5-38% (Sbracia *et al.* 2002).

It has also been shown that sex chromosomes are preferentially located in the subacrosomal region and it is possible that the introduction of sperm that did not undergo the acrosome reaction in the oocyte cytoplasm, and with an intact sperm peri-nuclear theca, may lead to an impaired decondensation of chromatin in the subacrosomal area, particularly for sex chromosomes, which are more frequently located there.

Sbracia et al performed a study to see if the increased rate of sex chromosome abnormalities in ICSI babies was due to the location of the sex chromosomes in the subacrosomal region or due to in increased rate of sex chromosomal aneuploidy in sperm. They found 40.9% of X chromosomes and 52.9% of Y chromsomes were localised in the subacrosomal region of the sperm nucleus compared with only 14.3% of chromosome 18. However, it is more likely that the presence of sex chromosomal aneuploidy in the sperm of oligospermic men has a greater influencing effect than the lack of sex chromosome decondensation.

There was also an increase in sex chromosome aneuploidy in the sperm of oligospermic men (2.9% v 0.7% controls). These sperm may be injected into an oocyte during the ICSI process producing an embryo with sex chromosome aneuploidy (Sbracia *et al.* 2002; Bonduelle *et al.* 1998a). Most cases of sex chromosome abnormality are compatible with life and therefore this process may lead to an increase in sex chromosome aneuploidy in ICSI infants.

Although sex chromosomal aneuploidy is not generally associated with congenital malformations, some individuals may have medical or educational problems. There is reportedly a higher incidence of temper tantrums in children with 47XYY and a higher rate of criminal behaviour in this group (Meschede and Horst 1997). However, the majority of individuals in the general population with sex chromosome abnormalities are never diagnosed and a prospective follow up study of children diagnosed with sex chromosomal abnormalities has shown that most lead completely normal lives (Ratcliffe 1999).

Turners syndrome 45X0 is one of the sex chromosome abnormalities that does have associated congenital malformations and medical problems (Jones 1988). Klinefelters syndrome XXY is often undiagnosed, but this condition is associated with a slightly lower mean IQ than the general population (Meschede and Horst 1997). Both of these syndromes are themselves associated with infertility, although there are case reports of Klinefelter fathers conceiving using the ICSI process (Poulakis *et al.* 2001).

1.8.3 Transmission of other genetic defects

Male infertility is associated with up to 10 times increased likelihood of chromosomal and genetic abnormalities and probably many as yet unidentifiable anomalies (Cummins and Jequier 1995; Bonduelle *et al.* 2002). Obstructive azoospermia may result from conditions such as congenital absence of the vas deferens; 50% of these men demonstrate the dF508 mutation of cystic fibrosis and are thus able to pass this gene onto their children conceived after ICSI (de Kretser 1995). Other similar syndromes causing epididymal obstruction and thus infertility, such as Young's syndrome (de Kretser 1995; Handelsman *et al.* 1984) are thought to be inherited disorders, but the involved genes are not known. This means that as well as increasing the possibility of passing on known genetic defects and increasing the risk of certain conditions, there is also the possibility of transmitting as yet unknown genetic defects, which could lead to abnormalities in the offspring.

Disorders of sperm motility are often due to deficiencies of specific proteins and may be associated with disorders of ciliary motility such as Kartagener's syndrome with bronchiectasis and dextrocardia. Sperm motility may also be disordered because of abnormal development of the outer dense fibres or the midpiece of the sperm, which is likely to arise from genetic mutations for these structural proteins (de Kretser 1995).

1.9 Genomic Imprinting

Genomic imprinting is the mechanism that determines the expression or repression of genes from maternal or paternal chromosomes. This modification of genetic material is epigenetic i.e. reversible between generations and is not a mutation. Maternal and paternal germlines confer an imprint or sex-specific mark on certain chromosome regions. Therefore although the sequence of the genes on these chromosomes could be identical, they are not functionally equivalent.

It is thought that imprinting is created by DNA methylation. This methylation process is known to be involved in regulating the activity of genes in different cell types. For most genes, the methylation patterns on the maternally and paternally derived chromosomes are indistinguishable. However, for the small number of genes subject to imprinting, the methylation pattern differs between the maternal and

paternal gene copy. The imprint is reset in the germline of each generation i.e. in the maternal germline, the paternal imprint on the paternal chromosome is switched to maternal.

This means that the genomes of primordial germ cells have had the previous imprint erased and after gonadal differentiation there is new methylation / imprinting of the developing germ cells according to the sex of the parent. The spermatocyte's genome has more methylation than the oocyte's. Following fertilisation of an egg by the sperm, both genomes undergo general demethylation with the exception of a small number of sites within the imprinted genes. Thereafter, during the pre-gastrula stage, cell-specific methylation patterns are established which, along with the imprinted patterns, are stably inherited through subsequent cell divisions.

Over 40 imprinted genes have now been characterised. They have been shown to influence embryonic growth and development and are implicated in the inactivation of tumour suppressor genes resulting in some childhood cancers e.g. Wilms tumour, embryonal rhabdomyosarcoma, osteosarcoma and bilateral retinoblastoma. These are thought to occur by the "two-hit" hypothesis of cancer. The first inactivation of a tumour suppressor allele would occur by imprinting rather than mutation. Wilms tumour appears to have two different tumour precursor lesions. One type is thought to be due to an imprinting defect of the gene for insulin-like growth factor-2 (IGF2). The second subtype occurs after a mutation of the WT1 gene (Reeve *et al.* 2002).

There is evidence that several syndromes are also caused by imprinting including Prader-Willi, Angelmans, Russell-Silver, transient neonatal diabetes, Beckwith-Wiedemann, Pseudohypoparathyroidism and McCune-Albright syndrome. There has been much work studying the chromosome deletions that occur in these syndromes.

Imprinting may be disrupted during the embryonal preimplantation period when demethylation, remethylation and imprint maintenance occurs. Since imprinted genes are frequently involved in growth and differentiation of the embryo, disruption of imprinting may lead to retardation of growth, arrested growth (and death) or overgrowth. This may explain why overgrowth or macrosomia is a feature of Beckwith-Wiedemann syndrome (De Rycke *et al.* 2002a; De Rycke *et al.* 2002b).

The process of assisted conception leads to manipulation of gametes, (which may or may not be functionally sub-optimal), and, usually, fertilisation in-vitro as well as culture of pre-implantation embryos. There are concerns that the process of imprinting could be disrupted during these procedures (Manning *et al.* 2001).

Two recent studies have suggested that there may an increased incidence of Beckwith-Wiedemann syndrome after assisted conception (DeBaun *et al.* 2003; Maher *et al.* 2003). Beckwith-Wiedemann occurs spontaneously as a result of uniparental disomy or epigenetic alterations at specific loci. Maher reported six cases of Beckwith-Wiedemann syndrome in children conceived with IVF or ICSI. Of the children who had molecular genetic studies performed, there were no cases of uniparental disomy. Two cases showed a loss of methylation that has previously been associated with monozygotic twinning, which is also associated with IVF/ICSI (Abusheikha *et al.* 2000).

Although small, these studies support previous findings. Olivennes et al reported a boy with Beckwith-Wiedemann in a cohort of 73 children conceived after IVF (Olivennes *et al.* 2001). An earlier study by Sutcliffe et al in 1995 also reported a child with Beckwith-Wiedemann in a cohort of 91 children born after replacement of frozen embryos (Sutcliffe *et al.* 1995). Work in animals also suggests that culture of pre-implantation embryos may be associated with abnormalities of growth. The culture-induced large offspring syndrome observed in cattle and sheep associated with fetal overgrowth and disproportionate enlargement of internal organs, resembles Beckwith-Wiedemann syndrome in humans (McEvoy *et al.* 1998; Khosla *et al.* 2001).

Angelmans syndrome is caused by a loss of the maternal allele function secondary to uniparental disomy of the paternal allele, a mutation of the maternal allele, or a sporadic genetic imprinting error causing a paternal imprint on a maternal chromosome (Cox *et al.* 2002). A report of two children with Angelmans syndrome, conceived after ICSI, suggested that an inherited defect was unlikely in these cases and therefore the defect was possibly caused at a post-zygotic stage.

The maternal methylation imprint on human chromosomes appears to occur after ovulation at or around the time of fertilisation (El-Maarri 2001). The ICSI process leads to mechanical stress and exposure of the oocyte to chemicals that could potentially affect the imprinting processes.

1.10 Behavioural studies and family relationships

Several studies have attempted to examine the parent-child relationships in IVF families. However, most are limited by small numbers, difficulties in matching socio-demographic factors and poor response rates to questionnaires. Where questionnaires are used, it is possible that IVF families may wish to portray a more positive picture in order to "prove" that they are good parents (Golombok *et al.* 2001).

The reasons that individuals give for wanting to have children have been compared between IVF and naturally conceived families (Colpin *et al.* 1998), but retrospective studies may not provide an accurate picture. The motives given for wanting to become a parent may be quite different for infertile couples that have not conceived when compared with couples that have conceived successfully.

Colpin et al examined parenting motives using the parenthood motivation list (van Balen and Trimbos-Kemper 1995). This is a self-reported questionnaire with subscales for:

- happiness - expected feelings of happiness in relationship with child
- well-being – expected positive effects for the family
- identity – desire to have children as means of achieving adulthood and strengthening identity
- motherhood – expectation that motherhood will give life-fulfilment
- continuity – desired affective relation with grown-up children and wish to live on though children after death
- social control – implicit or explicit pressure from outside the couple to procreate

Thirty one IVF families were compared with naturally conceived children at age 24-30 months. The groups were not matched for parental age or socio-demographic

factors, but a regression analysis was performed to adjust for these factors. For both groups happiness and family wellbeing were the most important motives for becoming a parent. However, IVF mothers had stronger motivation scores for identity, motherhood and social control. The social control motive was explained by the increase in age of IVF mothers and the authors comment that this supports the idea that social pressure to have children increases with the age of a woman.

The adjustment to parenthood was measured using the Questionnire for Parental Attitudes and Emotions (Colpin *et al.* 1998). There were few correlations between parenthood motives and adjustment to parenthood, but identity and motherhood motives correlated with anxious over concern. Motherhood and social control motives correlated with a more strained relationship with the child. Conversely, happiness and motherhood correlated with less strained relationships in naturally conceived families.

Researchers have suggested that IVF parents have more emotional involvement with and warmth towards their child (Golombok *et al.* 1996) and less parenting stress (Hahn 2001; Greenfeld *et al.* 1996; Golombok *et al.* 2001; Hahn and DiPietro 2001; van Balen 1996). Despite this there is some evidence of parental overprotection towards children (Golombok *et al.* 2001; McWhinnie 1996; Hahn and DiPietro 2001), higher stress and anxiety in the families (McMahon *et al.* 1997b; van Balen *et al.* 1996), and lower parental self-esteem (Gibson *et al.* 2000a; McMahon *et al.* 1997b).

1.10.1 Perceptions of treatment and pregnancy

Parents of IVF children have often had a long wait before conceiving. The investigative procedures and treatments are invasive and research into the psychological and physical burden of fertility treatment and the experience of pregnancy and delivery suggests that there is a higher degree of anxiety and stress in IVF parents compared to other parents during this period (van Balen *et al.* 1996; McMahon *et al.* 1997a).

Van Balen studied and compared three groups of parents (van Balen *et al.* 1996):

- Infertile parents who had conceived after IVF

- Previously infertile parents i.e. parents who were thought to be infertile, but who eventually conceived naturally (at least 4 years between the first attempt to conceive and the birth of the first child)
- Parents of naturally conceived children.

Questionnaires were sent to 65 IVF couples, 67 formerly infertile couples and 100 normally fertile couples. The response rate for men and women were 62% and 69% for the IVF group, 49% and 52% for the infertile group and 28% and 35% for the fertile group. This large difference was assumed to be due to a courtesy effect, whereby parents of IVF children were willing to cooperate with the unit which had helped them conceive.

In the IVF and previously infertile groups, mothers and fathers both recorded the psychological burden of the treatments as greater than the physical. There was no significant difference between these two groups with respect to the burden of treatment. The IVF and previously infertile mothers reported a higher level of complications during pregnancy. This appeared to be related to the age of mothers, which was highest in the IVF group and lowest in the fertile group. There were no significant differences in the frequency of complications in delivery. This does not reflect most other research, which indicates higher rates of complications in IVF families, but medical judgements were not taken into consideration and the results are solely from parental reports. IVF or previous infertile couples perceived the delivery to be a more exceptional event than fertile mothers.

Although there were no reported differences in the enjoyment of pregnancy, the IVF and previously infertile groups found the pregnancy to be a more stressful event than the control group. In an Australian study, McMahon et al also found IVF mothers to be more anxious about the well-being of their babies and about possible damage to the baby during childbirth in a study of anxiety and the quality of attachment to the fetus at 30 weeks of pregnancy in 70 IVF couples and 63 matched controls (McMahon *et al.* 1997a).

Macmahon et al also found IVF mothers to be more anxious about separation from the baby at birth. These anxieties were predominant in those mothers who had experienced 2 or more treatment cycles. However, women who had experienced one cycle of treatment and reported lower levels of anxiety were also found to have high levels of suppressed anxiety measured by biophysical and biochemical responses to provoked behavioural stressors. There was no difference between groups for fetal attachment or anxiety traits. The authors suggested that the reported increase in anxiety about the child outcome in the IVF group was not unreasonable as it is well recognised that IVF children are more likely to be born premature and to spend time on a neonatal unit.

Van Balen et al reported that fathers of children conceived after IVF found the pregnancy more exceptional and enjoyed their wives' pregnancy more than the other groups and considered that the medical treatments were more worthwhile than did the group of previously infertile fathers. The author suggested that this implied a greater degree of involvement of IVF fathers in the pregnancies of their partners. However, no group found the delivery itself to be a more exceptional experience (van Balen *et al.* 1996).

1.10.2 Parent-child relationships

Van Balen categorised the potential assumptions about parent–child relationships following IVF into four groups (van Balen 1996).

a) Parents with long-standing infertility may regard their IVF children as an extra special gift and therefore overprotect and show exaggerated concern and emotional involvement.
b) Children conceived after IVF may be extremely desired by their parents. This may lead to an over- expectation of their child's level of achievement
c) Parental stresses may increase as the adaptation to the commitment and concern for their child may be difficult after a long period of childlessness.
d) The child's behaviour may be altered if parental attitudes and behaviour are altered due to the perception that society will regard their IVF child as different from naturally conceived children.

Van Balen tested these four assumptions using Dutch psychology questionnaires. Three groups of families were compared: 2 groups of infertile couples (one group who conceived after IVF and a second group who conceived naturally after a minimum of 4 years) and a group of couples who had naturally conceived and had no history of infertility. Each family consisted of a mother, father and child aged 2-4 years. Parental response to these questionnaires varied, possibly due to the differences in motivation between the groups. The parents of IVF children had the highest response rate and the families of the normally fertile couples had the lowest. The numbers of families involved was small overall with a total of 115 mothers and 101 fathers taking part

Both the initially infertile mothers and IVF mothers reported significantly ($p=0.01$) stronger feelings toward their child and also reported that they experienced more pleasure in their child than did the normally fertile mothers. The IVF mothers and initially infertile mothers also reported more parental competence than fertile mothers ($p=0.012$). IVF mothers reported that their children were less obstinate and more social than the mothers of the other two groups. All differences remained significant when maternal age and the child's gender were included as co-variates. However, there were no significant differences amongst the fathers of the three groups for any of the variables assessed. This may indicate that women are more negatively affected by infertility than their husbands or it may reflect a greater degree of involvement of mothers.

The level of parental concern and stress was no higher in parents who had conceived after IVF. There was no significant difference between groups for emphasis on achievement i.e. the levels of parental expectation for the child's performance, particularly in comparison with others. A measure for parental burden (the degree to which the child prevents the parent giving attention to others i.e. their partner) was also similar between groups . This study therefore concluded that the experience of infertility may be beneficial for parent-child relationships in that it appears to be more warm and affectionate.

The earliest age at which parent-child interaction has been assessed was at 4 months postpartum by McMahon et al in a continuation of their longitudinal study

(McMahon *et al.* 1997b). The quality of mother-child interaction was assessed by videotaped studies of mother-baby interaction using "the still face procedure". Infant behaviour in response to a mother sitting opposite the infant with a still face has been shown to be related to the security of mother-child relationship at 12 months (Cohn *et al.* 1991). The IVF babies were found to be more "fussy" in response to the still face procedure and generally had more difficult temperaments. This was verified by an observer. After the still face procedure, the mother resumed play with her infant. No difference in quality of interaction between mothers and their children was found between groups.

There were no differences noted between the group of IVF mothers and their controls for anxiety, marital satisfaction, use of support services or postnatal depression. However, despite these findings, IVF mothers reported lower self-esteem. The authors suggest that indicated that some IVF mothers may have higher self-expectations. However, the difference in infant behaviour and fussiness cannot be fully explained by high maternal expectation. It has been suggested that anxiety in pregnancy may lead to a physiological effect on infant behaviour.

Raoul-Duval et al studied psychological development and mother-infant bonding in 33 IVF conceived children compared with two equal matched control groups: a group of 33 infants born to mothers who had ovulation induction, but no other assisted fertilisation and a group of 33 infants who were naturally conceived. Children were initially assessed at 9 months and an increase in sleeping difficulties was found amongst the IVF group. This correlated with lower maternal mood. At 18 months, the maternal depressive symptoms were declining and, although there was still an increase in sleeping difficulties in IVF children, the mothers complained less about this and appeared less anxious (Raoul-Duval *et al.* 1993).

This improvement in anxiety over time was also described in the cohort of Australian mothers and children discussed above (McMahon *et al.* 1997a), when re-studied at 12 months (Gibson *et al.* 2000b; Gibson *et al.* 2000a). The IVF mothers continued to report lower self-esteem, but these reports were much weaker than in the 4 month postpartum study, suggesting improvement. Parental attitudes regarding attachment to their children were comparable between the groups. There was no difference in the

level of parental stress or anxiety about separation, but IVF mothers saw their children as more vulnerable and special than did control mothers.

IVF fathers reported lower marital satisfaction and lower self esteem, but no difference in the experience of parenthood, attachment or attitudes to parenting. Golombok found that assisted reproduction fathers interacted more with their children and contributed more to parenting than fathers of naturally conceived children (Golombok *et al.* 1996).

A European study (Golombok *et al.* 1996) aimed to investigate family relationships in families of IVF children, donor insemination (DI) children, adoptive children and naturally conceived children. Four countries were involved, but there were only just over 100 children aged 4-8 in each group. IVF parents were found to interact more with their children and IVF mothers reported more emotional involvement and less stress. There was no difference in the children's perceptions of their family relationships or evidence of psychological disorders.

Families were followed up at the age of 11-12 years (Golombok *et al.* 2002). One hundred and two IVF families were compared to donor insemination, adoptive families and naturally conceived children. Parents and children were interviewed separately and questionnaires were completed to assess marital state, parental-child relationships and child behaviour. Assisted reproduction parents showed greater warmth and emotional involvement overall than adoptive and natural conception parents, although there was some evidence of an increase in over-concern and over-protectiveness in this group. The IVF children had positive psychosocial involvement i.e. parents and teachers reported no evidence of emotional or behavioural problems. Assisted reproductive children were equally verbally aggressive, but less physically aggressive than naturally conceived peers. Parents in assisted reproduction families seemed to have stable marriages and were psychologically healthy. There was a high level of warmth between parents and children with appropriate discipline and the children were well-adjusted.

Although these studies and others (Cederblad *et al.* 1996; Montgomery *et al.* 1999) suggest little difference in family relationships, particularly as the children get older

(Gibson *et al.* 2000a; Raoul-Duval *et al.* 1993), further studies have found that mothers of assisted reproduction children appeared to be over-concerned and overprotective of their children. In a small study, McWhinnie et al found that all parents of 43 IVF and GIFT children regarded themselves as overprotective (McWhinnie 1996). Greenfeld et al sent questionnaires to IVF families. There was a low response rate (31%). Mothers were more likely to reply than fathers. 52% mothers reported special feelings of attachment causing difficulty with separation compared with 19 % fathers (Greenfeld *et al.* 1996).

Colpin found that working mothers of IVF children allowed their child less autonomy and were more intrusive (Colpin *et al.* 1995). These mothers reported working more for economic reasons than for professional development and the authors hypothesised that these mothers would rather stay at home with their children. They therefore compensated for their absence by being more intrusive. This contrasts work by Gibson et al, which found no difference in professional activity (Gibson *et al.* 2000b).

Some of the Colpin et al study children were followed up at age 8-9 years (Colpin and Soenen 2002). They found that there was no significant differences between IVF parents and those that had been conceived naturally using measures of parenting stress, parenting and child behaviour and most parenting goals. The parenting goals assessed were: adjustment (to be kind and obedient in the relationship with parents); personal autonomy; achievement and social success; and religion (the extent to which the families are committed to a religion). Religion was more important to parents in the IVF group – perhaps a reflection of the fact that IVF families were recruited from a catholic hospital. However, fathers of IVF children also attached a greater importance to the "adjustment" goal than control fathers i.e. there was a greater expectation of the child to be obedient and to conform to parental expectations.

Colpin et al also found that although teacher ratings of the child's behaviour did not differ significantly between groups, there was an increase in the total problem behaviour scores for IVF children (Colpin and Soenen 2002). This contradicts work by Hahn and DiPietro who included a teacher questionnaire in their study of family

relationships in 3-7 year olds (Hahn and DiPietro 2001). The teachers of the children, blind to the mode of conception, rated IVF children as having fewer behavioural problems than naturally conceived controls, despite the mothers in the IVF group having less satisfaction with aspects of family functioning. Teachers also rated mothers of IVF children as having greater warmth, but not as being overprotective.

1.10.3 Disclosing the child's mode of conception

There has been little work investigating the number of families that disclose their child's method of conception to friends, family or the child him/herself. McWhinnie found in a study of 43 IVF and Gamete intrafallopian transfer (GIFT) children, that many parents were concerned that their child may be singled out and this led to various degrees of secrecy. Four out of 31 families had not even shared the conception method with their immediate family (McWhinnie 1996).

Greenfeld et al reported that families had requested help with the issue of disclosure to the child, regarding the manner of their conception; 25% remained uncertain whether to tell their child (Greenfeld *et al.* 1996).

Colpin et al studied 27 IVF families when the child was 8-9years old (Colpin and Soenen 2002). Seven (26%) had informed their child of the IVF conception. Sixteen (59%) reported that they intended to tell their child and three families were unsure. One family was certain that they did not want to tell their child. IVF families who had told their child reported more problem behaviours in their child compared to IVF families who had not yet told their child. This contrasts to work by Golombok which found that children conceived using donor insemination, who knew their conception method, had less frequent and severe disputes with their mothers than those who had not been told (Golombok *et al.* 1996).

Chapter 1

KEY POINTS

- **The first IVF child was born in 1978**
- **The first ICSI child was born in 1992**
- **Rapid development of new techniques continues without thorough evaluation of existing methods**
- **IVF/ICSI parents are more likely to be older, from a higher social class and Caucasian compared with other parents**
- **Replacement of single embryos decreases the incidence of multiple births and many of the associated perinatal complications**
- **There are few developmental outcome studies of IVF/ICSI children and they often lack size, power and adequate selection and matching of control groups**
- **There is no convincing evidence that there are any childhood developmental difficulties caused by the IVF/ICSI process per se**
- **The incidence of congenital malformations may be increased after IVF/ICSI**
- **ICSI conceived children are more likely to have minor chromosomal abnormalities**
- **A sub-group of male factor infertility may be passed onto ICSI conceived sons**
- **The incidence of genetically inherited conditions may be increased after IVF/ICSI**
- **Family relationships may differ in IVF/ICSI families compared with other families**

CHAPTER 2: AIMS AND OBJECTIVES

2.1 Background of study

There has been a relatively small amount of research into the outcomes of ICSI children. Most of the studies that have been reviewed in Chapter 1 involve infants and toddlers. Although ICSI was developed in 1992, the oldest ICSI children in the UK at the time of this study were mostly around the age of five years. Few studies have involved naturally conceived controls and a conventional IVF conceived children comparison group.

Much of the work for the study was a continuation of work by Sutcliffe et al comparing the physical and developmental outcome of ICSI and naturally conceived children at 18 months (Sutcliffe *et al.* 2001). This follow-up study involved the repeat assessment of children conceived after ICSI and natural conception at school entry and, in addition, this study included a comparison group of children conceived after conventional IVF techniques.

Part of the work for this thesis contributed to the UK arm of an International collaborative study. There were five main collaborators: Dr Sutcliffe (UK) and Dr Bonduelle (Begium) were the lead investigators working with Dr Wennerholm (Sweden), Dr Loft (Denmark) and Dr Tarlatzis (Greece). The UK arm of the study contributed one third of the total number of children assessed. Two scientific papers have been published using the data from this thesis (Bonduelle *et al.* 2005;Ponjaert-Kristoffersen *et al.* 2005).

The study was funded by the European Union Fifth Framework Quality of Life Programme. The stated scientific question for the study was: "Are ICSI-conceived children physically, neurodevelopmentally and emotionally normal at school entry, and has there been any demonstrable adverse impact on family relationships associated with the type of conception?"

The role of the candidate (Dr Catherine Peters) was to recruit families into the study and organise the assessments. As the main paediatrician involved in assessing the children, Dr Peters examined the majority of the study children and recorded the

physical examination findings and interviewed the parents. Dr Peters also supervised the psychological assessments performed by Miss Xenya Chrysostomou.

In addition to the child and family development study, two other studies are included in this thesis. Firstly, a survey of parental attitudes towards disclosing the mode of conception to their IVF conceived child. Secondly, a survey of children with Beckwith-Wiedemann syndrome to ascertain if there is an increase in conception after IVF techniques in this group of children compared to the general population.

2.2 Supervision

This thesis was jointly supervised by:
Dr Alastair Sutcliffe, Senior Lecturer and Honorary Consultant at the Royal Free Hospital, Medical School, University College London.
Professor Brent Taylor, Professor of Community Child Health, Royal Free Hospital Medical School, University College London.

2.3 Child and family development study

2.3.1 Hypotheses

Firstly, children born using ICSI are expected to display the following in comparison with both IVF and naturally conceived children:

- a greater occurrence of minor physical / congenital anomalies
- no difference in overall physical health
- a greater occurrence of fine and gross motor difficulties
- a greater occurrence of difficult temperament and emotional or behavioural problems

Secondly, families of children born using ICSI will experience the following:

- more commitment to parenting and lesser commitment to employment
- more stress in the parent-child relationship
- more stress in the marital relationship
- more mental health problems in parents

2.3.2 Objectives of study

The primary objectives of this study were to:

- assess the extent to which exposure to ICSI is associated with significant health, developmental and psychosocial adjustment outcomes at age of school entry
- determine the extent to which these outcomes are related to conception type once confounding factors are taken into account
- assess the effect of ICSI intervention on the levels of stress in the family, the parents psychological well being, and the parents attitude toward the child and family life in general
- determine the extent to which these outcomes are related to conception type once confounding and mediating factors are considered.

Secondary objectives - Subanalyses

- Enumeration of congenital anomalies
- Assessment of outcome differences with respect to sperm origin

2.4 Parental attitudes survey

- informing children about their mode of conception

2.4.1 Hypothesis

- Parents of children conceived after IVF are reluctant to inform their child about their mode of conception.

2.4.2 Objectives of study

The primary objectives of this study were to investigate IVF parents to:

- Establish the proportion of IVF parents who intend to inform (or have informed) their children of their mode of conception.
- Ascertain if parents who disclose their child's mode of conception to those outside the nuclear family are more likely to inform their own child.
- Assess if parents who have children conceived by different methods (IVF or ICSI) are more or less likely to inform their children of their mode of conception.

2.5 Genomic imprinting survey

2.5.1 Hypothesis

- Children conceived after in-vitro fertilization techniques are at increased risk of developing conditions caused by genetic imprinting defects (eg Beckwith-Wiedemann syndrome) than naturally conceived children.

2.5.2 Objectives of study

The primary objective of this study was:

- To determine if children conceived after assisted reproduction techniques have an increased risk of conditions arising from defects in genomic imprinting using the example of Beckwith-Wiedemann syndrome.

CHAPTER 3: CHILD AND FAMILY DEVELOPMENT STUDY
-STUDY DESIGN AND METHODS

3.1 Study Type

A population case-control study of school aged children (and their families) born after Intracytoplasmic Sperm Injection (ICSI), conventional in-vitro fertilisation (IVF) or natural conception. The study involved a total of 510 children living throughout the United Kingdom. Children were grouped according to the mode of their conception with 189 children conceived after ICSI, 158 children conceived after conventional IVF and 163 naturally conceived children. Children were seen at a chronological age between 4 years 6 months and 5 years 6 months between November 2000 and September 2002.

3.1.1 Geographical area

Children were recruited from fertility clinics in England and Scotland. Several children and families resided in Wales, but the clinics in Wales were not performing ICSI at the time that this cohort of children was conceived. This meant that families had to attend clinics in England. Children were invited to attend appointments for the assessment at one of eight centres; Royal Free Hospital, London; CARE at the Park Hospital, Nottingham; NURTURE at Queens Medical Centre, Nottingham; St Mary's Hospital, Manchester; Leeds General Infirmary; Aberdeen Maternity Hospital; Ninewells Hospital, Dundee; private premises in Bristol, affiliated to Bristol IVF clinic.

3.1.2 Power of study

The power of the study was calculated for the total European collaboration. The power for the UK arm of the study is therefore reduced with 189 UK children in the ICSI group and 163 control children. The WPPSI-R subscales have a standard deviation of 15 and taking a 5-point difference between any two groups as significant the UK cohort would have 88% power (at a two-sided significance of 5%) to detect such a difference between control and ICSI children. The McCarthy subscales have a standard deviation of 10 and to detect a 5-point difference, the UK cohort would have over 99% power (at a two-sided significance of 5%) to detect such a difference between control and ICSI children.

The main European study was powered to detect a twofold difference in the rates of major congenital anomalies between the control children and the ICSI children (where it was assumed that the birth prevalence among control children was 4%). Thus, in order to have 80% power at a two-sided significance level of 5% to detect such a risk difference, it was calculated that 603 children would be needed in each group. Because the data presented in this thesis are based on only a subgroup of the data however (i.e. children from the UK), the power of these data to detect such a risk difference is substantially lower. In retrospect, with 189 UK children in the ICSI group and 163 control children, the power of this subgroup to detect a twofold difference in major congenital anomaly rates would be only 27% (39% if a one-sided test had been used).

3.1.3 Statistical analyses

All analyses were performed using SAS version 8.2 (Cary Institute, NC). Continuous measurements are expressed as mean (95% confidence interval for the mean) and categorical measures as n; % (95% confidence interval). For prevalence, exact confidence intervals were calculated in preference to confidence intervals based on the normal approximation to the binomial distribution. Tests for differences in mean levels of continuous measurements between the three study groups were assessed by one way analysis of variance. Differences in the prevalence of categorical measures between the 3 groups were assessed using Pearson's chi-square test or, if cell counts were low (<5), using Fisher's exact test. All statistical tests were two-sided and a p-value of <0.05 was interpreted as "statistically significant".

3.1.4 Ethical approval

Ethical approval was obtained from the London Multicentre Research Ethics Committee on the 22nd September 2000. The reference number for the study is MREC/98/2/1

3.2 Method of recruitment

3.2.1 ICSI children

The majority of children conceived after ICSI were recruited from a cohort of 208 children seen by Sutcliffe for the first time in their second year of life (Sutcliffe *et al.* 2001). This group of children had been recruited from fertility clinics throughout

England and Scotland during 1997-1999. The clinics contacted all eligible families using a standard letter and families were able to reply and opt in to the study if they wished. An average reply rate of 90% was achieved.

In addition to this group of children, we also included 12 families who contacted us when we were seeking to recruit conventional IVF conceived children.

3.2.2 Naturally conceived children / control group

This group of children were recruited using several methods. Firstly, 221 families of naturally conceived children participating in the previous cohort study by Sutcliffe were re-contacted and invited to participate in this follow up study.

This group had been originally recruited using three methods:

a) Parents of ICSI-conceived children were invited to bring a "buddy" ie a child who was of the same age and sociodemographic matching.
b) Nurseries associated with fertility centres were asked to help recruit matched children.
c) Two London general practices were asked to help recruit matched children who were due for their eighteen-month developmental surveillance assessment.

In addition, first-born, caucasian, five year olds were contacted through schools. A standardised letter (see appendix 1) was sent to schools willing to participate and the letter was distributed to children between the ages of 4 years 6 months and 5 years 6 months. The parents were then able to reply and opt-in to the study if they wished. Those that replied were checked to ensure that the matching criteria were met and if so, they were entered into the study.

3.2.3 IVF children / comparison group

This comparison group was not used in the study of eighteen month old ICSI children by Sutcliffe et al. Therefore all children were newly recruited through participating fertility clinics. A standard letter of invitation to join the study (see appendix 2) was sent to all eligible children and families were able to opt-in to the study if they chose to do so.

3.2.4 Non-attenders and drop out rates

The original cohort of ICSI conceived children who participated in the study by Sutcliffe included 90% of those families contacted. The non-responders from the largest three centres, NURTURE in Nottingham, Midland Fertility Service and the Lister in London were compared with participating families and no significant sociodemographic differences were found.

When the previously participating families were re-contacted for this study, the drop out rate for the ICSI cohort of children was recorded (see Chapter 4 - results). Where possible the reasons for refusal/withdrawal were evaluated and recorded.

The number of naturally conceived control children from the original cohort included in this study was reduced because of the differences in eligibility and matching criteria (see 3.2.5). Of the remaining eligible children, the drop out rate was recorded and the reasons for drop out were evaluated.

3.2.5 Matching

Children and families were matched for age, sex, race, gestational maturity (equal or greater than 32 weeks) and maternal parity. All children were singleton.

3.2.6 Benefits of Assessment

Families who agreed to take part in the study were given an opportunity to discuss medical and behavioural concerns with the paediatrician and psychologist at the time of the assessment. The families were also advised that they could contact the investigators at any time in the future if there were any further concerns of a paediatric nature, regardless of the relevance to the study per se.

During the course of the assessment, previously unrecognised medical problems were sometimes discovered. This allowed early referral and treatment where necessary.

3.3 The Assessment

A paediatrician (mainly the candidate*) and a psychologist (Miss Xenya Chrysostomou) assessed all the children during joint assessments using a standardised protocol. The psychologist was "blinded" to the mode of conception of the children. The children were assessed by the psychologist in a child-friendly environment whilst the parents were interviewed by the paediatrician in an adjacent room. Once the child had completed the assessment by the psychologist, he/she was then examined by the paediatrician. The child's parents were present during this physical assessment.

After the assessment, information about sperm parameters for fathers of ICSI children was obtained from an existing database and recorded. Where this information was missing, the clinics were contacted (with paternal consent) and the information was requested.

After each joint assessment the questionnaire data was recorded on a laptop computer using the Filemaker Pro computer software program. This laptop data was also backed up regularly onto the medical school mainframe computer. At a later date, all the data was entered for a second time onto a second computer database. This achieved 100% double data entry. The data recording was compliant with the European data protection laws

* Some of the children assessed at the beginning of the study were seen by Dr Sutcliffe, Dr Shein, Dr Swan, Dr Joffe and Dr Ahmad, pending the appointment of the candidate, Dr Peters. The assessments were supervised and therefore standardised by Dr Sutcliffe. It was impossible to test; retest the assessments in order to standardise further. All the children were assessed by the same psychologist, Miss Chrysostomou.

The paediatrician completed a general medical questionnaire during the interview with the parents. This included sociodemographic details and past medical history including perinatal, neonatal and childhood data. The paediatrician also recorded all physical findings on this form. The psychologist recorded assessment data on standardised result sheets for each test which facilitated the scoring and recording of data.

.3.4 General Medical Questionnaire (see appendix 3)

3.4.1 Sociodemographic details

For each participating biological parent the following details were obtained:

- Age
- Marital status
- Educational level - divided into: no qualifications; GCSE/O-levels/CSE; A-levels; University entry or equivalent exams; Graduate degree; and Postgraduate degree.
- Occupation
- Social class was then obtained using the Standard Occupational Classification produced by the Office of Population Censuses and Surveys
- Smoking
- Drinking

In addition maternal gravida and parity were recorded and maternal height. Fathers were asked if they had children from previous relationships.

3.4.2 Perinatal details

Details of maternal illnesses, obstetric complications and medications taken during pregnancy were recorded. Parents were asked if initial ultrasound scans suggested the presence of more than one fetus, and if there were twins on scan at 20 weeks.

Mothers were asked if they smoked or drank alcohol during the pregnancy and if so, to quantify the amount. Alcohol consumption was recorded as the number of units consumed per week

Other data obtained included mother's intention to breastfeed, whether mother worked during the pregnancy and the number of persons providing adult support for mother during the pregnancy.

Details of the delivery included the date of delivery; the gestation at delivery in weeks and days; whether the labour commenced spontaneously or required induction or, as in some cases, there was no labour (elective caesarian section); and the type of

delivery - classified as normal vaginal, forceps/ventouse, planned section, emergency section, unknown.

3.4.3 Neonatal details

Records of birth weight, birth length, birth head circumference and apgar scores were obtained from the parent held child health record. If the data was missing, the parents were asked if they had other records from which this information could be obtained.

Records were made of the need for resuscitation at birth and for admission to the neonatal unit. If neonatal unit admission was required, then the length of stay and the reason for admission were recorded. The need for and length of ventilation was recorded. Neonatal illnesses were recorded using a defined list and congenital anomalies were classified according to the WHO international classification of disease (ICD-10). Abnormalities were also classified as major if they caused impairment or required surgical intervention.

The type of feeding was recorded as exclusive breast feeding, formula or mixed. The length of exclusive breast feeding was noted. The number of adult support for the baby and family was recorded including parents.

3.4.4 Childhood details

Details of childhood illnesses, the need for surgery and the number of hospital admissions were recorded. Details of medications and any other therapies ie physiotherapy, speech and language therapy were recorded.

3.5 Physical examination

3.5.1 General physical examination

A general medical examination was performed on all the children. It included cardiovascular, respiratory, ear nose and throat, abdominal and skin assessment. Neurological examination including gross motor skills was also performed. Attention was also paid to the dentition, hair and skeleton. The genitalia were examined in the male children. Testicular descent was checked for, and if the child was circumcised, the reason for this was recorded.

3.5.2 Congenital abnormalities

The children were examined carefully for evidence of congenital abnormalities and if found, theses were recorded according to the International Classification of Diseases (ICD-10). They were also classified as major and minor according to the definition of a major abnormality as "an abnormality causing impairment or requiring surgical intervention" (Bonduelle *et al.* 2002). Minor anomalies were recorded according to Aase: Textbook of Diagnostic Dysmorphology (Aase 1990).

3.5.3 Growth

All children were measured for height, weight and head circumference. Height was measured using a portable stadiometer recommended by the Child Growth Foundation. Weight was measured using metric electronic scales. Centiles for height and weight were then calculated using a computer programme (L-grow®), standardised for a UK cohort of children (LGROW 1998).

3.5.4 Audiometry

Each child had hearing testing performed using a portable pure tone audiometer. Results were recorded as normal, abnormal, no cooperation, difficult to assess, not done. Normal hearing was defined as lowest hearing level between 0-20dB under optimal conditions (IntercomptexUK 1993). If hearing was abnormal, the lowest level of hearing was recorded. Parents were also advised to arrange a formal hearing test if any abnormality was suspected.

3.5.5 Vision

Children were examined for visual acuity using the Snellen chart, evidence of squint using the cover test and range of eye movement. Stereo vision was assessed using a stereo test (StereoTest 1994). It consisted of 3 tests to check stereoscopic depth perception. The first image was of a large house fly, which establishes the presence of gross stereopsis. The second test allows the child to select a forward appearing animal from a selection of animal images. This allows some grading of fine depth discrimination. Finally the child was asked to select one out of four circles that is forward appearing. This allows finer discrimination assessment. Results were recorded as normal or abnormal.

3.6 Sperm classification

Data was recorded for sperm parameters of fathers of ICSI children. The cause of the male factor subfertility was also recorded. The definitions used were in concordance with the WHO classification:

- Normal spermatozoa >20,000,000 per ml, >50% normal motility and >30% normal head forms.
- Teratozoospermia <30% spermatozoa with normal head morphology
- Asthenozoospermia <50% spermatozoa with forward progression
- Oligozoospermia <20 million spermatozoa per ml

Information was collected regarding the sperm source for treatment and results of any testing for karyotype, testicular biopsy and/or cystic fibrosis carrier status. This information was held in a database created by Dr Sutcliffe during his assessment of ICSI children aged 12-24 months (Sutcliffe *et al.* 2001). The same data was requested from the appropriate clinics for the fathers of the 12 newly recruited ICSI children. The data collection form is shown as appendix 4.

3.7 Psychological assessments

3.7.1 Wechsler Preschool and Primary Scale of Intelligence (Weschler 1990)

This is an individually administered assessment of intelligence designed for children aged from 3 years to 7 years and 3 months and last standardised in the UK in 1989.

There are two distinct subsets – Performance (perceptual-motor) and Verbal. The division has been made to allow for the child's response to tasks. The Performance tasks require motor responses such as pointing, placing or drawing and the Verbal subtests require spoken responses.

Ten subsets have been used; five Performance and five Verbal. The subtests used are illustrated in tables 3.1 and 3.2.

At the end of each subtest, a raw score is generated. These scores are then individually converted to scaled scores using tables appropriate to the child's age. The sum of the scaled scores is then used to find the full IQ using a table of equivalents. This gives an IQ equivalent of the sum of scaled scores for verbal subsets, performance subsets and the full scale.

Table 3.1: WPPSI Performance Subtests.

Object assembly:	The child is presented with pieces of a coloured puzzle arranged in a standardised configuration. The child has to complete the puzzle in a specified time. This is repeated for five more puzzles.
Block design:	The child is required to reproduce patterns made from flat two-coloured blocks within a specified time.
Mazes:	The child is required to solve printed mazes using a pencil. Each maze becomes progressively harder.
Picture completion:	The child must identify what is missing from pictures of common objects that are presented to them.
Animal pegs:	The child is given a board with rows of 4 squares. Each square contains a hole for a peg and a picture of an animal. At the top of the board, each of the 4 animals are represented by a different coloured peg. The child is given a number of coloured pegs and the assessor demonstrates how the pegs must be placed according to the animal shown and the colour that represents it.

Table 3.2: WPPSI Verbal Subtests.

Information	The child has to demonstrate knowledge about objects and events in the environment. The child is presented with a stimulus booklet and is asked a question. The child is required to point to the correct object in a group of several objects ie *look at these pictures. Point to the one you cook on.* The remaining test requires the child to give verbal responses to questions ie *How many ears do you have?*
Comprehension	The child must explain the reasons for actions and the consequences of events ie *what would happen if you touch a hot oven?* The answer is scored on the quality of the response.
Arithmetic	The child must demonstrate knowledge and understanding of basic quantitative concepts. Firstly, he/she must point to one out of several objects in the stimulus book that demonstrates a qualitative concept ie *which on is the longest?* They must then demonstrate an ability to count and manipulate blocks. Lastly the child must solve verbally presented arithmetic problems ie *Sue has 3 apples. She eats 2 of them. How many does she have left?*
Vocabulary	In the first subtest, the child must name the object in a picture. In the second part, the child must give a definition for orally presented words ie *what is a knife?* The answer is scored on the quality of the response.
Similarities	This has 3 subtests. In the first part the child has to choose from one group of pictures the one object that is similar to another group of pictures. The second part the child must complete verbal sentence by showing an understanding of the similarity concept in the sentence ie *You wear shoes and you also wear...?* Response*: socks, trainers or item of clothing.* The third subtest asks the child to explain how 2 orally presented concepts are similar ie *How are a spoon and a fork alike?* The answer is scored on the quality of the response

3.7.2 McCarthy Motor Scales of Children's Abilities (McCarthy 1972)

This is one of a selection of scales of children's abilities. It has been designed for use in children aged between 2½ and 8½. The motor scale assesses a child's coordination when performing gross and fine motor tasks. There are five subtests: leg coordination, arm coordination, imitative action, draw-a-design and draw-a-child. These tests are described in table 3.3.

Each test is individually scored and the sum of the scores of the five subtests provides the composite raw score. Using a table of equivalents the raw score can be converted to a scale index appropriate to the exact age of the child. This index has a mean of 50 with a standard deviation of 10.

Table 3.3: McCarthy Motor scales

Leg coordination	The child is asked to perform six tasks, which are scored according to the quality of the performance. The examiner explains and demonstrates the tasks. Maximum points are obtained if the child can: Walk backwards for up to 5 steps Walk on tiptoes for a distance of up to 2 feet Walk in a straight line along a 9-foot tape on the floor Stand on one foot for up to 10 seconds Stand on the other foot for up to 10 seconds Skip rhythmically using alternate feet
Arm coordination	The child is asked to perform six tasks, which are scored according to the quality of the performance. The examiner explains and demonstrates the tasks. Maximum points are obtained if the child can: Bounce the ball with palm of hand up to 15 bounces Catch a beanbag thrown from 9 foot away (up to three times in a row) Catch a beanbag with one hand when thrown from 9 feet away (up to three times in a row) Catch a beanbag with the other hand when thrown from 9 feet away (up to three times in a row) Throw a beanbag with one hand through a target hole from a distance of 6 feet (up to three times in a row) Throw a beanbag with the other hand through a target hole from a distance of 6 feet (up to three times in a row)
Imitative action	The child is asked to imitate the following actions: Crossing feet at the ankles Folding hands on table with fingers intertwined Fold hands as above and twiddle (rotate) thumbs about each other Look through a plastic tube with one eye
Draw-a-design	The child copies designs in a booklet. The designs are scored using the examiners demonstration booklet
Draw-a-child	The child is asked to draw a picture of a girl or boy. The picture is scored according to criteria set out in the examiners booklet. The greater the detail of body parts and their positioning the higher the score.
Laterality	The examiner notes which hand the child prefers to catch and throw a beanbag with ie which hand they use first. For the ball bouncing, draw-a-design and draw-a-child the examiner notes if the child uses their right, left or both hands. From these scores the examiner can evaluate whether or not the child has established a hand dominance and with which hand. The eye the child looks through the tube with is also recorded

3.7.3 Bene-Anthony Family Relations Test (Bene and Anthony J 1978)

This assessment tests the child's positive and negative feelings towards his/her family members and the child's perception of positive and negative feelings from the family members towards himself/ herself. It also measures the feelings of dependency on others.

The child is initially asked two questions. Firstly, *"Who are the people who live with you at home?"* and secondly, *"Who are the people in your family?"* After the child has named these people, they may choose figures to represent the significant family members including self, mother, father, siblings and others. There is also a figure named "nobody" that represents the feelings that cannot be attributed to the other figures. Each figure has a box-like base with a slit at the top. Each question posed by the assessor is written on small cards and the child is asked to post the card into the figure that the message on the card fits best.

The test is scored by counting the total of positive and negative feelings attributed to each figure. The child's dependence on family figures can also be scored.

3.8 Parental questionnaires to assess family functioning

The biological parents of all participating children were asked to complete seven questionnaires relating to family functioning. The parents were given the questionnaires to complete at home and a reply paid envelope was provided. Parents were asked not to confer. The psychologist scored the returned questionnaires and the numerical scores were added to the computer database. If the questionnaires were not returned, a reminder was sent to the parents with a second copy of the questionnaires. The response rates of mothers and fathers were recorded.

If one parent did not have regular contact with the child, they were individually excluded, but the remaining parent was asked to complete their own questionnaires. Parents who were separated were not given the Dyadic Adjustment Scale of stress in the marital relationship. Copies of the questionnaires are provided in appendix 5.

3.8.1 The Child Behaviour Checklist 4-16 years: CBCL (Achenbach 1991)

This is a parental report of behavioural psychopathology in the children. The list contains 118 items for difficult behaviour, scored on a 3-point Likert scale. Principal components analyses revealed eight categories: withdrawn (rather be alone, shy), somatic complaints (dizzy, headaches), anxious or depressed (cries, nervous), social difficulties (acts young, not liked), thought difficulties (hears or sees things, repeats acts), attention difficulties (cannot concentrate, impulsive), delinquent behaviour (steals, swears), and aggressive behaviour (mean, threatening).

These components are scored into 3 groups: internalising behaviour (anxious, somatic, withdrawn behaviour), externalising behaviour (aggressive and delinquent behaviour) and social competence (social, thought, and attention problems). A total behaviour problem score is calculated.

3.8.2 The McDevitt/Carey Temperament Scale (McDevitt and Carey 1978)

This questionnaire measures the temperament of 3-7 year old children. The "temperament" is the behavioural style of the child in interaction with the environment and is of importance in personality development. The questionnaire responses provide information on nine dimensions of temperament and also categorises children as easy (rhythmic, approaching, adaptable, mild and positive), difficult (arrhythmic, low in approach and adaptability, intense and predominantly negative in mood) or intermediate.

3.8.3 The Parenting Stress Index/Short Form (Abidin 1990)

This questionnaire is based on the theory that the three measured subscales influence parenting behaviour.

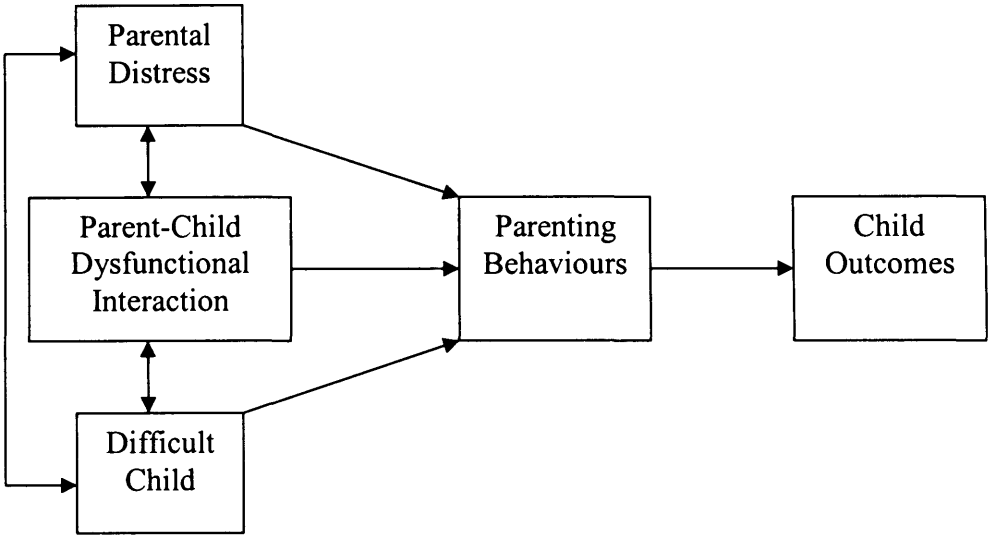


Figure 3.1 Theoretical model for the Parental Stress Index (Abidin 1990)

The questionnaire includes 36 items with a 5-point agree/disagree response scale. The responses measure the three subscales of Parental Distress, Parent-Child Dysfunctional Interaction and Difficult Child behaviour. The sum of these three scores represents the total parenting stress score. Parents with a total score above 90th percentile have clinically significant levels of stress.

This questionnaire also includes a defensive reporting scale. This is to assess possible parental bias in their responses. A parent with an extremely low score is either trying to portray a very competent individual or is detached in their role as a parent or is an extremely competent parent.

3.8.4 The Parental Acceptance and Rejection Questionnaire (McGuire and Earls 1993)

This questionnaire is a measure of parental behaviour with acceptance (warmth) at one end and rejection at the other. Warmth can be expressed physically and verbally and rejection (or the absence of warmth and affection) may be experienced as aggression/hostility or neglect/indifference.

The questionnaire includes 60 statements describing a parent's attitude toward their child and there is a 4-point Likert-type response scale to measure how true these statements are perceived to be by the parent. The total score represents acceptance-rejection and four subscales describe warmth, aggression / hostility, neglect and rejection.

3.8.5 Parenting and Work Commitment (Greenberger and Goldberg 1989)

There are two aspects to this questionnaire – a work commitment scale and a parenting commitment scale. These have been designed to assess the degree to which work commitments influence parenting commitment and behaviour towards the child. The work commitment is a 17-item scale, with items rated on a 6-point scale from Strongly Disagree to Strongly Agree. A total score is obtained and constructs covered are centrality of work to the self, psychological salience of work relative to other activities, and level of career aspirations. Non-working parents are omitted from this subscale, but are scored on the parenting commitment scale. The parenting commitment scale is of the same format and also has 17 items. Total score incorporates centrality of parenting to the self, psychological salience of parenting, and level of aspirations as a parent.

3.8.6 The Dyadic Adjustment Scale (Spanier 1976)

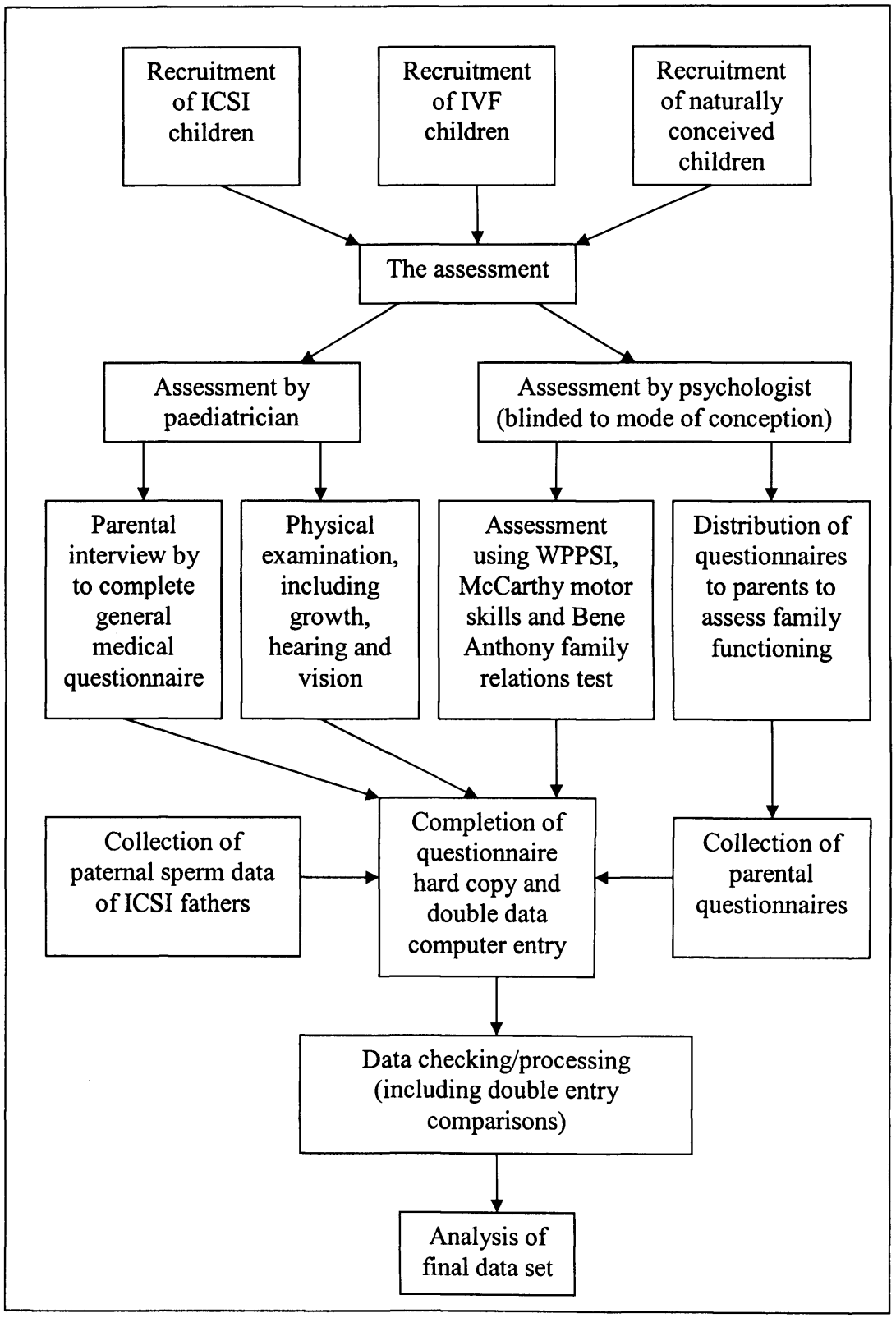
This measures the quality of the parental relationship. This is a 32-item scale for married or unmarried cohabiting couples and measures four subscales: dyadic satisfaction, dyadic consensus (ie level of agreement in lifestyle matters), dyadic cohesion (ie engaging in ideas and activities together) and expression of affection.

3.8.7 The General Health Questionnaire: GHQ-28 (Goldberg and Hillier 1979)

This is a self-administered 28-item screening instrument designed to detect current mental health problems in the general population. The GHQ-28 provides four dimensions, measuring somatic symptoms, anxiety and insomnia, social dysfunction, and severe depression.

Chapter 3

SUMMARY OF ASSESSMENT



CHAPTER 4: CHILD AND FAMILY DEVELOPMENT STUDY

- RESULTS AND DISCUSSION OF PHYSICAL EXAMINATION AND NEURODEVELOPMENT

4.1 Children studied

189 ICSI conceived children, 158 IVF conceived and 163 naturally conceived children were assessed. 177 ICSI children and 111 controls were recruited from the assessment performed by Sutcliffe et al at aged 18 months (see chapter 3). The remainder were newly recruited, as were all the IVF children.

4.1.1 Drop out rate

Children who participated in the previous study were invited to participate in this study if eligible. In total 110 control children and 31 ICSI children did not participate in this follow up assessment. 42 controls and 6 ICSI children were not eligible because of age, ethnicity, and birth order or because their conception status was known / revealed to the psychologist (ie psychologist was not blinded).

Other reasons for non-participation were: the family had moved abroad; the family had moved and were not traceable; the family were unwilling to travel in order to be seen; the family did not attend mutually arranged appointments; the family declined, but the reasons for not doing so were either not given or were not clear. Of the original cohort of children, reasons for non-participation are shown in figures 4.1 and 4.2.

After assessment, 4 control children, 11 IVF children and 0 ICSI children were excluded because of child refusal or the child was found to ineligible at the point of assessment. In the latter cases the assessment was carried out for the interest of the parent.

4.1.2 Recruitment of naturally conceived controls

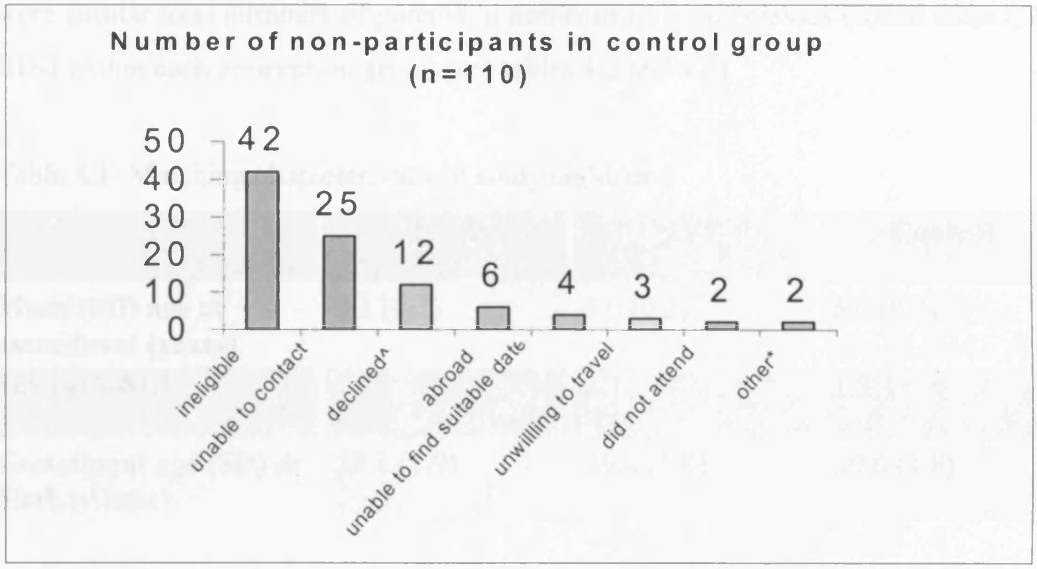
Letters to the headmasters/headmistresses of approximately 100 London primary schools were sent out asking for help to recruit naturally conceived children. Most schools did not wish to be involved with the study because of time restraints on their staff. However, the schools that did participate were asked to give recruitment letters to parents of children aged between $4^1/_2$ and $5^1/_2$. Replies were received from 193

parents of control children. 102 children were ineligible, mainly because of ethnicity, age and birth order. In addition, one family was uncontactable, one family subsequently declined to be involved in the study and one family did not attend several mutually arranged appointments. Of the remaining 88 eligible children, 56 newly recruited controls were then assessed in the order in which they could be contacted. It was not possible to assess the remaining 32 children within the time limits of the study.

4.1.3 Recruitment of the IVF comparison group

IVF children were recruited from 8 IVF centres across the UK. 158 eligible children were assessed. In addition, a further 40 families of IVF children offered to participate: 25 children were ineligible; 6 were uncontactable; 2 refused to travel for the assessment; 5 families did not attend mutually arranged appointments; 1 mother was due to give birth to twins at the time the child was eligible for assessment; 1 child's parents were in the process of divorce and no longer wished to be involved in the study.

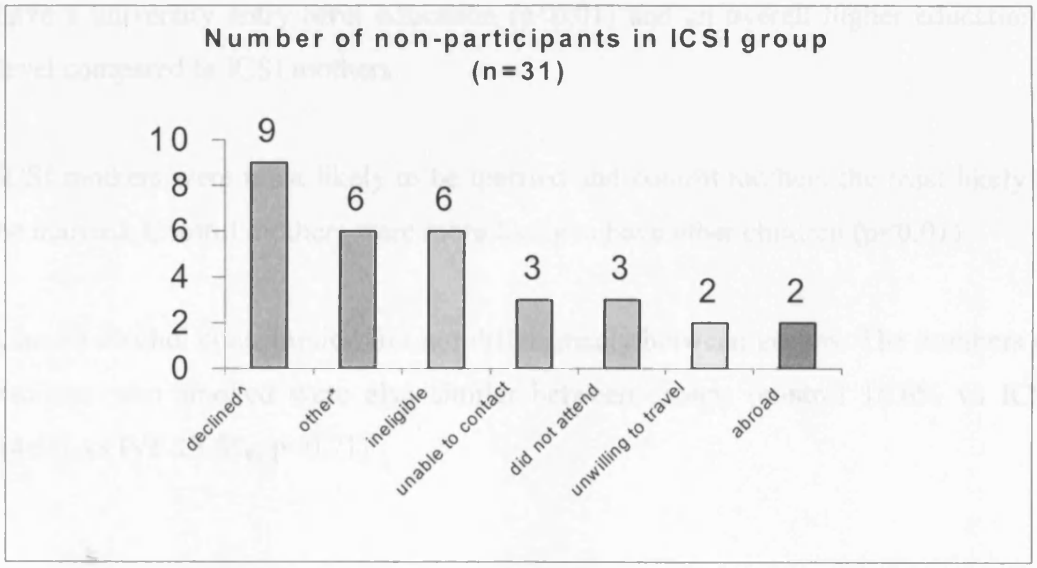
Figure 4.1 Reasons for non-participation in control group



*Reasons given included: child had mutism (1); child had stage 3 neuroblastoma (1).

^Reason for decline not established

4.2 Reasons for non-participation in ICSI group



*Reasons given include: Recent death of father (1); maternal ill health (2); child had neuroblastoma (1); child had glycerol carnase deficiency (1); parents in dispute with fertility clinic (1).

^Reason for decline not established

4.2 Matching

Study children were well matched for age, sex, gestational maturity (table 4.1). There were similar total numbers of parents in non-manual social classes (social class I, II, IIIN) within each conception group (see tables 4.2 and 4.3)

Table 4.1: Matching characteristics of study children

	ICSI	IVF	Control
Mean (SD) age at assessment (years)	5.1 (0.3)	5.0 (0.3)	5.0 (0.3)
Sex ratio M:F	1.3:1	1:1	1.3:1
Gestational age (SD) at birth (weeks)	39.1 (1.9)	39.4 (1.9)	39.6 (1.8)

4.3 Maternal sociodemographic data (table 4.2)

Mothers of ICSI and IVF children were older than mothers of control children (mean age: ICSI 34.4 vs IVF 35.1 vs control 31.7; $p<0.001$). There was no difference in social class between maternal groups. Control group mothers were more likely to have a university entry level education ($p<0.01$) and an overall higher educational level compared to ICSI mothers.

ICSI mothers were most likely to be married and control mothers the least likely to be married. Control mothers were more likely to have other children ($p<0.01$).

Current alcohol consumption did not differ greatly between groups. The numbers of mothers who smoked were also similar between groups (control 16.6% vs ICSI 14.3% vs IVF 13.5%; $p=0.71$)

Table 4.2 Maternal sociodemographic details

	ICSI mothers (n=189)		IVF mothers (n=156)		Control mothers (n=163)		p [§]
	No.	**% (95% CI)**	**No.**	**% (95% CI)**	**No.**	**% (95% CI)**	
Maternal age in years (mean (95% CI))		34.4 (33.9 - 34.9)		35.1 (34.5 - 35.7)		31.7 (30.9-32.5)	**<0.001**
Maternal social class							0.161
1	24	12.7 (8.3 - 18.3)	18	11.5 (7.0 – 17.6)	20	12.3 (7.7 – 18.3)	
2	71	37.6 (30.6 - 44.9)	63	40.4 (32.6 – 48.5)	78	47.9 (40.0 – 55.8)	
3N	66	34.9 (28.2 - 42.2)	43	27.6 (20.7 – 35.3)	40	24.5 (18.2 – 31.9)	
3M	22	11.6 (7.4 - 17.1)	23	14.7 (9.6 – 21.3)	14	8.6 (4.8 – 14.0)	
4	5	2.7 (0.9 - 6.1)	9	5.8 (2.7 – 10.7)	8	4.9 (2.1 – 9.4)	
5	1	0.5 (0.0 - 2.9)	0	0.0 (0.0 – 1.9)	3	1.8; (0.4 – 5.3)	
Maternal education							
-University entry exam or higher	81	42.9 (35.7 - 50.2)	62	39.7 (32.0 – 47.9)	92	56.4 (48.5 – 64.2)	
Subgroups:							**<0.01**
-higher degree	23	12.1 (7.9 - 17.7)	16	10.3 (6.0 – 16.1)	19	11.7 (7.2 – 17.6)	
-degree	36	19.1 (13.7 - 25.4)	39	25.0 (18.4 – 32.6)	53	32.5 (25.4 – 40.3)	
-university entry	22	11.6 (7.4 - 17.1)	7	4.5 (1.8 – 9.0)	20	12.3 (7.7 – 18.3)	
-A-level	26	13.8 (9.2 - 19.5)	23	14.7 (9.6 – 21.3)	19	11.7 (7.2 – 17.6)	
-GCSE / O-level	73	38.6 (31.7 - 46.0)	59	37.8 (30.2 – 45.9)	45	27.6 (20.9 – 35.1)	
-No qualification	9	4.8 (2.2 - 8.9	12	7.7 (4.0 – 13.1)	7	4.3 (1.7 – 8.7)	
Maternal smoking							
-current smoker	27	14.3 (9.6 – 20.1)	21	13.5 (8.5 - 19.8)	27	16.6 (11.2 – 23.2)	0.717

[§] The p-value corresponds to the global test of equality across all three treatment groups

Table 4.2 Maternal sociodemographic details (cont'd)

	ICSI mothers (n=189)		IVF mothers (n=156)		Control mothers (n=163)		*p*
	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	
Marital status							**<0.001**
-married	176	93.1 (88.5 – 96.3)	137	87.8 (81.6 – 92.5)	112	68.7 (61.0 – 75.7)	
-single	1	0.5 (0.0 – 2.9)	1	0.6 (0.0 – 3.5)	15	9.2 (5.2 – 14.7)	
-separated	7	3.7 (1.5 – 7.5)	6	3.9 (1.4 – 8.2)	12	7.4 (3.9 – 12.5)	
-divorced	0	0.0 (0.0 – 1.6)	0	0.0 (0.0 – 1.9)	1	0.6 (0.0 – 3.4)	
-living together	3	1.6 (0.0 – 4.6)	12	7.7 (4.0 – 13.1)	22	13.5 (8.7 – 19.7)	
-widow	2	1.1 (0.1 – 3.8)	0	0.0 (0.0 – 1.9)	1	0.6 (0.0 – 3.4)	
Maternal alcohol consumption							
-current drinker	122	67.0 (59.7 – 73.8)	114	73.6 (65.9 – 80.3)	103	64.8 (56.8 – 72.2)	0.221
Gravidity							0.404
-one	130	68.8 (61.7 – 75.3)	103	66.0 (58.0 – 73.4)	101	62.0 (54.0 – 69.4)	
-two or more	59	31.2 (24.7 – 38.4)	53	34.0 (26.6 – 42.0)	62	38.0 (30.6 – 46.0)	
Parity							**<0.01**
-one	167	88.4 (82.9 – 92.6)	141	90.4 (84.6 – 94.5)	126	77.3 (70.1 – 83.5)	
-two or more	22	11.6 (7.4 – 17.1)	15	9.6 (5.5 – 15.4)	37	22.7 (16.5 – 29.9)	

4.4 Paternal sociodemographic data (table 4.3)

Fathers of ICSI and IVF children were older than fathers of control children (mean age: IVF 36.0 vs ICSI 35.1 vs control 32.8; $p<0.001$). There was a trend for control fathers to be of higher social class ($p=0.09$) and to be more likely to have been educated to university entry level status (ICSI 45% vs IVF 42.3% vs control 54.6%; $p=0.07$ – not significant (NS)).

There was no significant difference between the numbers of children that the fathers in each group had from previous relationships. However, there was a trend towards ICSI fathers having two or more children from previous relationships.

Current alcohol consumption differed between groups, with IVF fathers most likely to drink and control fathers the least (IVF 87.7% vs ICSI 80% vs control 74.4%; $p<0.01$). Smoking was more common in the control group of fathers, and the number of fathers that smoked in the ICSI and IVF groups were similar (control 27.9% vs ICSI 16.9% vs IVF 15.4%; $p<0.01$).

In many cases the information regarding paternal sociodemographic factors was provided by the mother of the child. This meant that, in a few cases, the data was not available. Missing data is indicated in the tables.

Table 4.3 Paternal sociodemographic details

	ICSI fathers (n=189)		IVF fathers (n=156[a])		Control fathers (n=163[b])		*p* [§]
	No.	**% (95% CI)**	**No.**	**% (95% CI)**	**No.**	**% (95% CI)**	
Paternal age in years (mean (95% CI))		35.1 (34.4 - 35.8)		36.0 (35.2 - 36.8)		32.8 (32.0 - 33.6)	**<0.001**
Paternal social class							0.089
1	55	29.1 (22.7 – 36.1)	52	33.8 (26.4 – 41.8)	44	28.0 (21.2 – 35.7)	
2	73	38.6 (31.7 – 46.0)	56	36.4 (28.8 – 44.5)	73	46.5 (38.5 – 54.6)	
3N	10	5.3 (2.6 – 9.5)	9	5.8 (2.7 – 10.8)	7	4.5 (1.8 – 9.0)	
3M	41	21.7 (16.0 – 28.3)	33	21.4 (15.2 – 28.8)	19	12.1 (7.5 – 18.3)	
4	8	4.2 (1.8 – 8.2)	4	2.6 (0.7 – 6.5)	9	5.7 (2.7 – 10.6)	
5	2	1.1 (0.1 – 3.8)	0	0.0 (0.0 – 1.9)	5	3.2 (1.0 – 7.3)	
Paternal education							
-University entry exam or higher	85	45.0 (37.8 – 52.4)	66	42.3 (34.9 – 51.1)	89	54.6 (48.9 – 64.9)	
Subgroups:							0.065
-higher degree	21	11.1 (7.0 – 16.5)	10	6.5 (3.2 – 11.6)	21	13.5 (8.5 – 19.8)	
-degree	46	24.3 (18.4 – 31.1)	47	30.5 (23.4 – 38.4)	47	30.1 (23.1 – 38.0)	
-university entry	18	9.5 (5.7 – 14.6)	9	5.8 (2.7 – 10.8)	21	13.5 (8.5 – 19.8)	
-A-level	24	12.7 (8.3 – 18.3)	16	10.4 (6.1 – 16.3)	16	10.3 (6.0 – 16.1)	
-GCSE / O-level	67	35.5 (28.6 – 42.7)	62	40.3 (32.5 – 48.5)	41	26.3 (19.6 – 33.9)	
-No qualification	13	6.9 (3.7 – 11.5)	10	6.5 (3.2 – 11.6)	10	6.4 (3.1 – 11.5)	

[a] Data missing for 2 IVF fathers for social class and education

[b] Data missing for 7 control fathers for social class and education

[§] The p-value corresponds to the global test of equality across all three treatment groups

Table 4.3 Paternal sociodemographic details (cont'd)

	ICSI fathers (n=189 [a])		IVF fathers (n=156)		Control fathers (n=163 [b])		*p*
	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	
Paternal smoking							
-current smoker	32	16.9 (11.9 – 23.1)	24	15.4 (10.1 – 22.0)	44	27.9 (21.0 – 35.5)	**<0.01**
Paternal alcohol consumption							
-current drinker	148	80.0 (73.5 – 85.5)	136	87.7 (81.5 – 92.5)	116	74.4 (66.8 – 81.0)	**<0.05**
Children from previous relationships							
-none	165	87.8 (82.2 – 92.1)	141	90.4 (84.6 – 94.5)	147	91.9 (87.2 – 96.0)	0.282
-one	3	1.6 (0.3 – 4.6)	5	3.2 (1.1 – 7.3)	4	2.5 (0.6 – 6.3)	
-two or more	20	10.6 (6.6 – 16.0)	10	6.4 (3.1 – 11.5)	8	5.0 (2.2 – 9.7)	

[a] Data missing for 1 ICSI father for children from previous relationships

[b] Data missing for 4 control fathers for children from previous relationships

4.5 Perinatal results (table 4.4)

The number of illnesses / obstetric complications reported by mothers in pregnancy was highest in the IVF group and lowest in the control mother group (IVF 48.7% vs ICSI 42.3% vs control 35.0%; $p<0.05$). Infection during pregnancy, gastrointestinal illness and preterm labour occurred with similar frequency in each groups. Although pregnancy induced hypertension appeared to be less frequent in the control group, there was no significant difference between groups. (table 4.5).

Bleeding during pregnancy was more common in the ICSI and IVF group compared with the control group (ICSI 9.0% vs IVF 13.5% vs control 3.7%; $p<0.01$). Placenta praevia was more common in IVF and ICSI mothers ($p<0.001$) and ovarian hypertension syndrome was reported by some of the ICSI/IVF mothers (table 4.5).

The number of medications used in pregnancy was greater in the IVF and ICSI groups compared with control mothers (ICSI 44.4% vs IVF 42.3% vs control 31.9%; $p<0.05$). The differences were mainly accounted for by the use of progesterone by IVF and ICSI and mothers (table 4.6). The use of antibiotics, asthmatic drugs, allergy medication and vitamins was similar in each group.

There was no difference between groups for reported smoking in pregnancy. However, a significant difference was seen between maternal groups for alcohol consumption in pregnancy with ICSI and IVF mothers less likely to drink than control mothers (ICSI 8.0% vs IVF 7.1% vs control 17.8%; $p<0.01$).

There was a trend for IVF mothers to be more likely to work than ICSI and control mothers (IVF 80.1% vs ICSI 72.1% vs control 68.8%; p=0.07-NS). The numbers of adults available to support the mothers during pregnancy was also highest in the ICSI group and lowest in the control group ($p<0.001$). The intention of the mother to breastfeed was similar between groups at the time of pregnancy.

Twins were excluded from this study, but there was one mother in each of the ICSI and IVF groups who had been told that there was evidence that a second fetus had been present at the 20 week scan. Neither of these pregnancies resulted in twins. There was a large difference between groups for the reporting of a second fetus on

the first scan of pregnancy. ICSI and IVF mothers were much more likely to report a second fetus ($p<0.001$).

There were no differences between the groups for the spontaneous onset of labour or the induction of labour. Planned caesarian sections and the need for instrumental delivery (ventouse/ forceps) were also similar. However, Control mothers were most likely to have a normal vaginal delivery. ICSI and IVF mothers were more likely to have an emergency caesarian section than control mothers (post hoc testing: ICSI vs control $p=0.09$).

There is some missing data for perinatal results as indicated in table 4.4. The numbers involved are small, often missing data from a single child. This missing information is due to parental difficulty recalling specific information. In the case of one ICSI child, the mother had died and the father was unable to remember some of the details of the child's perinatal history. This also applies to the missing data for neonatal results in table 4.7.

4.6 Neonatal results (table 4.7)

Although the mean gestational age at birth was similar (+/- 5 days between groups) and represents close matching of groups, the difference was significant in view of the numbers of children involved in the study in each group. ICSI babies tended to be born earliest and control babies were born the most mature (gestational weeks: ICSI 39.1 vs IVF 39.4 vs control 39.6; $p<0.05$). There were no significant differences between groups for birth weight or head circumference.

ICSI children were less likely to need resuscitation at birth compared with the other groups (ICSI 5.3% vs IVF 9.0% vs control 11.0%). However, there was no significant difference seen between the three groups for resuscitation ($p=0.138$). A further direct comparison between ICSI and control groups showed a significant difference between these two groups for the need for resuscitation ($p<0.05$).

Ventilation occurred most in the control group, although very few babies required this in any group ($p=0.33$). The number of babies admitted to the neonatal unit was similar between groups, but the length of stay on the unit for these babies differed. If

all babies admitted to the neonatal unit are compared in terms of length of stay ≥7 days, then more ICSI babies remained on the unit for longer than 7 days compared with IVF and control babies (ICSI 68.4% vs IVF 38.9% vs control 27.8%; ICSI vs IVF p=0.07; ICSI vs control p<0.01). However, if the number of children who stayed on the neonatal unit for ≥7 days are considered in terms of the total number of children in each group, then this is no longer significant (p=0.25).

Reasons for admission to the neonatal unit included a range of common neonatal problems (see table 4.8). There was no difference seen between groups. The types of illnesses that affected the children as neonates were also varied (table 4.9). These illnesses did not necessarily require admission to the neonatal unit. Differences between groups were seen for sticky eye and cephalhaematoma, both of which were more prevalent in the IVF group.

Control mothers were most likely to exclusively breastfeed their babies than ICSI or IVF mothers (ICSI 58.2% vs IVF 44.2% vs control 64.2%; p<0.001). Of those mothers who chose to breast feed, the length of exclusive breast feeding was similar between groups.

The numbers of adults supporting the mothers after the birth of the babies was similar between groups with an average of 3 people (including the child's parents).

Table 4.4 Perinatal results

	ICSI (n=189[a])		IVF (n=156)		Control (n=163[b])		p [§]
	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	
Illness in pregnancy *	80	42.3 (35.2 – 49.7)	76	48.7 (40.7 – 56.8)	57	35.0 (27.7 – 42.8)	**<0.05**
Medications taken during pregnancy ^	84	44.4 (37.2 – 51.8)	66	42.3 (34.5 – 50.5)	52	31.9 (24.8 – 39.7)	**<0.05**
Alcohol consumption in pregnancy							
-current drinker	15	8.0 (4.5 – 12.8)	11	7.1 (3.6 – 12.3)	29	17.8 (12.3 – 24.5)	**<0.01**
Smoking in pregnancy	12	6.4 (3.3 – 10.8)	11	7.1 (3.6 – 12.3)	16	9.8 (5.7 – 15.5)	0.447
Work during pregnancy	119	72.1 (64.6 – 78.8)	125	80.1 (73.0 – 86.1)	99	68.8 (60.5 – 76.2)	0.068
Intention to breast feed							0.234
Yes	173	93.0 (88.3 – 96.2)	147	94.2 (89.3 – 97.3)	142	89.3 (83.4 – 93.7)	
No	13	7.0 (3.8 – 11.7)	9	5.8 (2.7 – 10.7)	17	10.7 (6.4 – 16.6)	
Adult support for mother							**<0.001**
-none	0	0.0 (0.0 – 1.6)	1	0.6 (0.0 – 3.5)	5	3.1 (1.0 – 7.0)	
-one	71	37.8 (30.8 – 45.1)	91	58.3 (50.2 – 66.2)	69	42.3 (34.6 – 50.3)	
-two or more	117	62.2 (54.9 – 69.2)	64	41.0 (33.2 – 49.2)	89	54.6 (46.6 – 62.4)	

* further details described in tables 4.5

^ further details described in tables 4.6

§ The p-value corresponds to the global test of equality across all three treatment groups

[a] Data missing for 3 ICSI children for intention to breast feed and for 1 ICSI child for adult support for mother

[b] Data missing for 4 control children for intention to breast feed

Table 4.4 Perinatal results (continued)

	ICSI (n=189[a])		IVF (n=156)		Control (n=163[b])		*p*
	No.	**% (95% CI)**	**No.**	**% (95% CI)**	**No.**	**% (95% CI)**	
More than one fetus on first USS							**<0.001**
Yes	32	17.4 (12.2 – 23.7)	32	20.5 (14.5 – 27.7)	3	1.9 (0.4 – 5.3)	
No	152	82.6 (76.3 – 87.8)	124	79.5 (72.3 – 85.5)	159	98.2 (94.7 – 99.6)	
More than one fetus at 20 weeks							0.763
Yes	1	0.5 (0.0 – 3.0)	1	0.6 (0.0 – 3.5)	0	0.0 (0.0 – 1.8)	
No	183	99.5 (97.0 – 100.0)	155	99.4 (96.5 – 100.0)	162	100.0 (98.2 - 100.0)	
Labour							0.321
-spontaneous	106	56.4 (49.0 – 63.6)	90	57.7 (49.5 – 65.6)	101	62.4 (54.4 – 69.8)	
-induced	58	30.9 (24.3 – 38.0)	41	26.3 (19.6 – 33.9)	47	29.0 (22.2 – 36.7)	
-no labour	24	12.8 (8.4 – 18.4)	25	16.0 (10.7 – 22.7)	14	8.6 (4.8 – 14.1)	
Method of delivery							0.202
-vaginal	87	46.3 (39.0 – 53.7)	68	43.6 (35.7 – 51.8)	90	55.2 (47.2 – 63.0)	
-forceps/ ventouse	33	17.6 (12.4 – 23.8)	30	19.2 (13.4 – 26.3)	31	19.0 (13.3 – 25.9)	
-planned caesarean section	22	11.7 (7.5 – 17.2)	19	12.2 (7.5 – 18.4)	19	11.7 (7.2 – 17.6)	
-emergency caesarean	46	24.5 (18.5 – 31.3)	39	25.0 (18.4 – 32.6)	23	14.1 (9.2 – 20.4)	
Gestational age at birth – weeks (mean (95% CI))		39.1 (38.8 - 39.4)		39.4 (39.1 - 39.7)		39.6 (39.3, 39.9)	**<0.05**

[a] Data missing for 5 ICSI children for presence of more than one fetus on first USS and 20 week ultrasound. Data missing for 1 ICSI child for labour and method of delivery.

[b] Data missing for 1 control child presence of more than one fetus on first USS and for onset of labour

Table 4.5 Illness in pregnancy/obstetric complications

	ICSI (n=189)		IVF (n=156)		Control (n=163)		p [§]
	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	
Pregnancy Induced Hypertension	11	5.8 (2.9 – 10.2)	11	7.1 (3.6 – 12.2)	5	3.1 (1.0 – 7.0)	0.264
Bleeding during pregnancy	17	9.0 (5.3 – 14.0)	21	13.5 (8.5 – 19.8)	6	3.7 (1.4 – 7.8)	**<0.01**
Placenta praevia	10	5.3 (2.6 – 9.5)	15	9.7 (5.5 – 15.4)	1	0.6 (0.0 – 3.4)	**<0.001**
Infection during pregnancy	7	3.7 (1.5 – 7.5)	6	3.9 (1.4 – 8.2)	6	3.7 (1.4 – 7.8)	0.996
Preterm labour	0	0.0 (0.0 – 1.6)	0	0.0 (0.0 – 1.9)	1	0.6 (0.0 – 3.4)	0.628
Ovarian cyst/ hyperstimulation syndrome	6	3.3 (1.2 – 6.8)	2	1.3 (0.2 – 4.6)	0	0.0 (0.0 – 1.8)	
Gastrointestinal illness	18	9.5 (5.7 – 14.6)	8	5.1 (2.2 – 9.9)	12	7.4 (3.9 – 12.5)	0.302
Any illness during pregnancy	80	42.3 (35.2 – 49.7)	76	48.7 (40.7 – 56.8)	57	35.0 (27.7 – 42.8)	**<0.05**

[§] The p-value corresponds to the global test of equality across all three treatment groups

Table 4.6 Medications in pregnancy

	ICSI (n=189)		IVF (n=156)		Control (n=163)		*p*
	No.	**% (95% CI)**	**No.**	**% (95% CI)**	**No.**	**% (95% CI)**	
Antibiotics	11	5.8 (2.9 – 10.1)	2	1.3 (0.2 – 4.6)	9	5.5 (2.7 – 10.7)	0.057
Asthmatic drugs	1	0.5 (0.0 – 2.9)	2	1.3 (0.2 – 4.6)	3	1.8 (0.4 – 5.3)	0.523
Allergy medication	2	1.1 (0.1 – 3.8)	1	0.6 (0.0 – 3.5)	0	0.0 (0.0 – 1.8)	0.645
Progesterone	32	16.9 (11.9 – 23.0)	21	13.5 (8.5 – 19.8)	0	0.0 (0.0 – 1.8)	**<0.001**
Vitamins / electrolytes	3	1.6 (0.3 – 4.6)	3	1.9 (0.4 – 5.5)	3	1.8 (0.4 – 5.3)	1.000
Iron	9	4.8 (2.2 – 8.9)	16	10.3 (6.0 – 16.1)	16	9.8 (5.7 – 15.4)	0.107
Antihypertensives	3	1.6 (0.3 – 4.6)	2	1.2 (0.2 – 4.6)	1	0.6 (0.0 – 3.4)	0.779
Antacids	13	6.9 (3.7 – 11.5)	9	5.8 (2.7 – 10.7)	12	7.4 (3.9 – 12.5)	0.844
Any medication	84	44.9 (37.2 – 51.8)	66	42.3 (34.4 – 50.5)	52	31.9 (24.8 – 40.0)	**<0.05**

Table 4.7 Neonatal characteristics

	ICSI (n=189[a])		IVF (n=156)		Control (n=163[a])		*p*
	No.	**% (95% CI)**	**No.**	**% (95% CI)**	**No.**	**% (95% CI)**	
Male	107	56.6 (49.2 – 63.8)	79	50.6 (42.5 - 58.7)	91	55.8 (47.9 - 63.6)	0.498
Birth weight – kgs (mean (95% CI))	-	3.26 (3.18 - 3.34)	-	3.32 (3.24 - 3.40)	-	3.33 (3.24 - 3.42)	0.413
Birth length – cms (mean (95% CI))	-	51.3 (50.8 - 51.8)	-	51.7 (51.2 - 52.2)	-	51.4 (50.9 - 51.9)	0.652
Birth head circumference – cms (mean (95% CI))	-	34.3 (34.1 - 34.5)	-	34.3 (34.1 - 34.5)	-	34.5 (34.2 - 34.8)	0.664
Resuscitation	10	5.3 (2.6 – 9.5)	14	9.0 (5.0 – 14.6)	18	11.0 (6.7 – 16.9)	0.138
Admission to neonatal unit	20	10.6 (6.6 – 15.9)	20	12.8 (8.0 – 19.1)	18	11.0 (6.7 – 16.9)	0.796
≥7 days on neonatal unit	13	6.9 (3.7 – 11.5)	7	4.6 (1.9 – 9.1)	5	3.1 (1.0 – 7.0)	0.248
Ventilation required	1	0.5 (0.0 – 2.9)	1	0.6 (0.0 – 3.5)	4	2.5 (0.7 – 6.2)	0.330
Intention to breast feed							0.234
Yes	173	93.0 (88.3 – 96.2)	147	94.2 (89.3 – 97.3)	142	89.3 (83.4 – 93.7)	
No	13	7.0 (3.8 – 11.7)	9	5.8 (2.7 – 10.7)	17	10.7 (6.4 – 16.6)	
Exclusively breastfed	110	58.2 (50.8 – 65.3)	69	44.2 (36.3 – 52.4)	104	64.2 (56.3 – 71.6)	**<0.001**
Length of exclusive breast feeding							
none	45	24.5 (18.4 – 31.3)	41	26.3 (19.6 – 33.9)	34	21.9 (15.7 – 29.3)	0.137
<4weeks	18	9.8 (5.9 – 15.0)	16	10.3 (6.0 – 16.1)	9	5.8 (2.7 – 10.7)	
-5-8 weeks	23	12.5 (8.1 – 18.2)	30	19.2 (13.4 – 26.3)	16	10.3 (6.0 – 16.2)	
-9-12 weeks	23	12.5 (8.1 – 18.2)	16	10.3 (6.0 – 16.1)	21	13.6 (8.6 – 20.0)	
>12 weeks	75	40.8 (33.6 – 48.2)	53	34.0 (26.6 – 42.0)	75	48.4 (40.3 – 56.5)	
Adult support for mother (> three adults)	62	32.8 (26.2 – 40.0)	48	30.8 (23.6 – 38.7)	60	36.8 (29.4 – 44.7)	0.505

[a] Data missing for 3 ICSI children for intention to breast feed and 5 ICSI children for length of exclusive breastfeeding.
[b] Data missing for 4 control children for intention to breast feed and 8 control children for length of exclusive breastfeeding.

Table 4.8 Reasons for admission to neonatal unit (NNU)

	ICSI (n=189)		IVF (n=156)		Control (n=163)		*p*
	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	
Feeding	10	5.4 (2.6 - 9.8)	4	2.6 (0.7 - 6.4)	6	3.7 (1.5 - 7.8)	0.429
Sepsis (including group B streptococcus)	2	1.1 (0.1 - 3.9)	1	0.6 (0.0 - 3.5)	1	0.6 (0.0 - 3.4)	1.000
Hypoglycaemia	1	0.5 (0.0 - 3.0)	3	1.9 (0.4 - 5.5)	1	0.6 (0.0 - 3.4)	0.454
Hypothermia	1	0.5 (0.0 - 3.0)	0	0.0 (0.0 - 1.9)	1	0.6 (0.0 - 3.4)	1.000
Infant of diabetic mother	0	0.0 (0.0 - 1.6)	1	0.6 (0.0 - 3.5)	0	0.0 (0.0 - 1.8)	0.307
Jaundice	2	1.1 (0.1 - 3.9)	2	1.3 (0.2 - 4.6)	0	0.0 (0.0 - 1.8)	0.472
Jittery	0	0.0 (0.0 - 1.6)	2	1.3 (0.2 - 4.6)	0	0.0 (0.0 - 1.8)	0.094
Low birth weight	2	1.1 (0.1 - 3.9)	0	0.0 (0.0 - 1.9)	1	0.6 (0.0 - 3.4)	0.779
Cardiac murmur	1	0.5 (0.0 - 3.0)	0	0.0 (0.0 - 1.9)	0	0.0 (0.0 - 1.8)	1.000
Prematurity	1	0.5 (0.0 - 3.0)	2	1.3 (0.2 - 4.6)	3	1.8 (0.4 - 5.3)	0.523
Respiratory difficulties	4	2.2 (0.6 - 5.5)	7	4.5 (1.8 - 9.0)	7	4.3 (1.7 - 8.7)	0.409
Bleeding (haemophilia)	0	0.0 (0.0 - 1.6)	0	0.0 (0.0 - 1.9)	1	0.6 (0.0 - 3.4)	0.628

Table 4.9 Neonatal illnesses

	ICSI (n=189)		IVF (n=156)		Control (n=163)		p [§]
	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	
Feeding difficulties							
-feeding difficulties	17	9.2 (5.5 – 14.4)	22	14.1 (9.1 – 20.6)	13	8.0(4.3 – 13.3)	0.153
-vomiting	2	1.1 (0.1 – 3.9)	1	0.6 (0.0 – 3.5)	0	0.0(0.0 – 1.8)	0.645
Respiratory difficulties							
-apnoea	2	1.1 (0.1 – 3.9)	2	1.3 (0.2 – 4.6)	1	0.6 (0.0 – 3.4)	0.868
-meconium aspiration	0	0.0 (0.0 – 1.6)	1	0.6 (0.0 – 3.5)	3	1.8 (0.4 – 5.3)	0.115
-pneumonia	1	0.5 (0.0 – 3.0)	0	0.0 (0.0 – 1.9)	1	0.6 (0.0 – 3.4)	1.000
-pneumothorax	0	0.0 (0.0 – 1.6)	0	0.0 (0.0 – 1.9)	1	0.6 (0.0 – 3.4)	0.628
-RDS	2	1.1 (0.1 – 3.9)	6	3.9 (1.4 – 8.2)	4	2.5 (0.7 – 6.2)	0.243
-ventilation	0	0.0 (0.0 – 1.6)	0	0.0 (0.0 – 1.9)	1	0.6 (0.0 – 3.4)	0.628
Infection							
-pyrexia	0	0.0 (0.0 – 1.6)	1	0.6 (0.0 – 3.5)	0	0.0 (0.0 – 1.8)	0.307
-septicaemia	3	1.6 (0.3 – 4.7)	1	0.6 (0.0 – 3.5)	1	0.6 (0.0 – 3.4)	0.632
-skin infection	1	0.5 (0.0 – 3.0)	2	1.3 (0.2 – 4.6)	2	1.2 (0.2 – 4.4)	0.741
-sticky eye	2	1.1 (0.1 – 3.9)	12	7.7 (4.0 – 13.1)	3	1.8 (0.4 – 5.3)	**<0.01**
-umbilical infection	2	1.1 (0.1 – 3.9)	0	0.0 (0.0 – 1.9)	0	0.0 (0.0 – 1.8)	0.334
-UTI	0	0.0 (0.0 – 1.6)	1	0.6 (0.0 – 3.5)	0	0.0 (0.0 – 1.8)	0.307
Hypoglycaemia	1	0.5 (0.0 – 3.0)	5	3.2 (1.1 – 7.3)	3	1.8 (0.4 – 5.3)	0.146
Hypothermia	3	1.6 (0.3 – 4.7)	3	1.9 (0.4 – 5.5)	1	0.6 (0.0 – 3.4)	0.641

[§] The p-value corresponds to the global test of equality across all three treatment groups

Table 4.9 Neonatal illnesses (cont'd)

	ICSI (n=189)		IVF (n=156)		Control (n=163)		*p*
	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	
Neurological							
-convulsion	1	0.5 (0.0 – 3.0)	0	0.0 (0.0 – 1.9)	0	0.0 (0.0 – 1.8)	1.000
-intracranial haemorrhage	0	0.0 (0.0 – 1.6)	0	0.0 (0.0 – 1.9)	1	0.6 (0.0 – 3.4)	0.628
-jittery	0	0.0 (0.0 – 1.6)	2	1.3 (0.2 – 4.6)	0	0.0 (0.0 – 1.8)	0.094
Blood transfusion	0	0.0 (0.0 – 1.6)	0	0.0 (0.0 – 1.9)	1	0.6 (0.0 – 3.4)	0.628
Jaundice	36	19.6 (14.1 – 26.0)	22	14.1 (9.1 – 20.6)	24	14.7 (9.7 – 21.1)	0.387
Birth trauma							
-cephalhaematoma	0	0.0 (0.0 – 1.6)	3	1.9 (0.4 – 5.5)	0	0.0 (0.0 – 1.8)	**<0.05**
-fracture	1	0.5 (0.0 – 3.0)	0	0.0 (0.0 – 1.9)	0	0.0 (0.0 – 1.8)	1.000
Delayed passage meconium	1	0.5 (0.0 – 3.0)	1	0.6 (0.0 – 3.5)	1	0.6 (0.0 – 3.4)	1.000
Cardiac							
-cardiac murmur	0	0.0 (0.0 – 1.6)	1	0.6 (0.0 – 3.5)	1	0.6 (0.0 – 3.4)	0.532
-cyanosis	0	0.0 (0.0 – 1.6)	0	0.0 (0.0 – 1.9)	1	0.6 (0.0 – 3.4)	0.628

4.7 Physical assessment (table 4.10)

Hospital admissions were similar between groups (table 4.10). Parents of IVF children reported the highest number of illness in their children compared to parents of ICSI and naturally conceived controls (p=0.08). Illnesses were classified into upper respiratory tract, lower respiratory tract, dermatological, gastrointestinal and other infections (table 4.11). Of these, there was a trend towards less illness in the control group for gastrointestinal and dermatological complaints (p=0.09). Childhood medications were similar between the groups (table 4.12).

The children in each group had similar need for therapies such as speech and language therapy, physiotherapy, child psychology (table 4.10). When general abnormalities on physical examination were compared, the only difference noted was an increase in adenopathy noted in the control group ($p<0.05$). There were no differences of abnormalities for the cardiovascular, respiratory, abdominal, dermatological or neurological systems. Genitourinary abnormalities were seen in boys in the ICSI and IVF groups, but not in the control group.

There was no significant difference between the three conception groups for incidence of surgery (table 4.13). However, there was a trend towards more surgery in the ICSI children (ICSI 16.9% vs IVF 14.7% vs control 10.4%). Genitourinary surgery (excluding circumcision) was most likely to have been performed in ICSI children compared to IVF and control (table 4.13).

4.8 Growth (table 4.14)

There were no differences noted between groups for childhood growth in terms of actual height in cms or centiles. Comparison of the children's growth was more meaningful using the centiles for height and weight, rather than absolute values. These centiles adjust for exact age and were calculated accurately using the L-grow® computer program standardized for the UK population of children (LGROW 1998). However, there is no accurate software to calculate head circumference centiles. The head circumference data was therefore based on raw measurement only, but no significant difference between head circumference was seen (p=0.07).

4.9 Audiology (table 4.15)

There was no difference in hearing test results between the children in all of the groups. There were similar numbers in each group who were unable to cooperate (refusal to place the audiometer over their ears for the whole assessment and/or poor concentration) and some children were difficult to assess. These children would appear to want to cooperate, but did not seem understand what was required. Of the children who were able to perform the audiometry testing, there were similar numbers of children who had abnormal results and were unable to hear sounds above the "normal" threshold of 20dB. No child was completely deaf. There were 4 ICSI children, 2 IVF children and 4 control children who refused to participate in this assessment.

4.10 Opthalmology (table 4.16)

No differences were found between groups for the frequency of strabismus, abnormal stereotactic vision or visual acuity. No child had abnormal eye movements. No child was registered blind or blind on examination.

Table 4.10 Physical development

	ICSI (n=189)		IVF (n=156)		Control (n=163)		p [§]
	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	
Admitted to hospital	59	31.2 (24.7 – 38.4)	49	31.4 (24.2 – 39.3)	47	28.8 (22.0 – 36.4)	0.852
Other therapies							
Speech and Language therapy	17	9.0 (5.3 – 14.0)	15	9.6 (5.5 – 15.4)	9	5.5 (2.6 – 10.2)	0.342
Physiotherapy	4	2.1 (0.6 – 5.3)	2	1.3 (0.1 – 4.6)	0	0.0 (0.0 – 1.8)	0.169
Child psychology	2	1.1 (0.1 – 3.8)	1	0.6 (0.0 – 3.5)	1	0.6 (0.0 – 3.4)	1.000
Any therapy	21	11.1 (7.0 – 16.5)	17	10.9 (6.5 – 16.9)	11	6.8 (3.4 – 11.8)	0.314
Cardiovascular abnormalities	4	2.2 (0.6 – 5.5)	5	3.2 (1.1 – 7.3)	2	1.2 (0.2 – 4.4)	0.513
Heart murmur	4	2.2 (0.6 – 5.5)	5	3.2 (1.1 – 7.3)	2	1.2 (0.2 – 4.4)	0.513
Femoral pulse	1	0.5 (0.0 – 2.9)	0	0.0 (0.0 – 1.9)	2	1.2 (0.2 – 4.4)	0.518
Respiratory abnormalities	0	0.0 (0.0 – 1.6)	0	0.0 (0.0 – 1.9)	0	0.0 (0.0 – 1.8)	-
Abdominal abnormalities	1	0.6 (0.0 – 3.0)	0	0.0 (0.0 – 1.9)	0	0.0 (0.0 – 1.8)	1.000
Palpable liver	0	0.0 (0.0 – 1.6)	0	0.0 (0.0 – 1.9)	0	0.0 (0.0 – 1.8)	-
Palpable spleen	0	0.0 (0.0 – 1.6)	0	0.0 (0.0 – 1.9)	0	0.0 (0.0 – 1.8)	-
Genitourinary abnormalities in boys	2	2.0 (0.1 – 3.9)	4	6.2 (1.7 – 15.0)	0	0.0 (0.0 – 4.1)	0.067

[§] The p-value corresponds to the global test of equality across all three treatment groups

Table 4.10 Physical development (cont'd)

	ICSI (n=189)		IVF (n=156)		Control (n=163)		*p*
	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	
Dermatological abnormalities							
Skin (excl birthmarks)	2	1.3 (0.1 – 3.8)	0	0.0 (0.0 – 1.9)	0	0.0 (0.0 – 1.8)	0.334
- Eczema present	8	4.2 (1.8 – 8.2)	5	3.2 (1.1 – 7.3)	13	8.0 (4.3 – 13.3)	0.121
Hair	1	0.5 (0.0 – 2.9)	0	0.0 (0.0 – 1.9)	0	0.0 (0.0 – 1.8)	1.000
Nails	0	0.0 (0.0 – 1.6)	0	0.0 (0.0 – 1.9)	0	0.0 (0.0 – 1.8)	-
Skeleton	1	0.5 (0.0 – 2.9)	1	0.6 (0.0 – 3.5)	0	0.0 (0.0 – 1.8)	0.761
Neurological abnormalities	2	1.1 (0.1 – 3.8)	1	0.6 (0.0 – 3.5)	0	0.0 (0.0 – 1.8)	0.646
Walk	2	1.1 (0.1 – 3.8)	0	0.0 (0.0 – 1.9)	0	0.0 (0.0 – 1.8)	0.334
Run	2	1.1 (0.1 – 3.8)	1	0.6 (0.0 – 3.5)	0	0.0 (0.0 – 1.8)	0.646
Jump	1	0.5 (0.0 – 2.9)	1	0.6 (0.0 – 3.5)	0	0.0 (0.0 – 1.8)	0.761
Other abnormalities							
Adenopathy	1	0.5 (0.0 – 2.9)	2	1.3 (0.2 – 4.6)	8	4.9 (2.1 – 9.4)	**<0.05**
Dentition	2	1.1 (0.1 – 3.8)	2	1.3 (0.2 – 4.6)	3	1.8 (0.4 – 5.3)	0.894
Ears	4	2.1 (0.6 – 5.3)	1	0.6 (0.0 – 3.5)	4	2.5 (0.7 – 6.2)	0.483
Mouth	1	0.5 (0.0 – 2.9)	0	0.0 (0.0 – 1.9)	1	0.6 (0.0 – 3.4)	1.000
Nose	0	0.0 (0.0 – 1.6)	0	0.0 (0.0 – 1.9)	0	0.0 (0.0 – 1.8)	-

Table 4.11 Childhood illnesses

	ICSI (n=189)		IVF (n=156)		Control (n=163)		*p*
	No.	**% (95% CI)**	**No.**	**% (95% CI)**	**No.**	**% (95% CI)**	
Upper respiratory tract	29	15.3; 10.5 – 21.3)	20	12.8; 8.0 – 19.1)	22	13.5; 8.7 – 19.7)	0.779
Lower respiratory tract	35	18.5; 13.3 – 24.8)	24	15.4; 10.1 – 22.0)	23	14.1; 9.2 – 20.4)	0.509
Dermatological[a]	37	19.6; 14.2 – 26.0)	41	26.3; 19.6 – 33.9)	27	16.6; 11.2 – 23.2)	0.090
Gastrointestinal	15	7.9; 4.5 – 12.8)	18	11.5; 7.0 – 17.6)	8	4.9; 2.1 – 9.4)	0.094
Other infections	16	8.5; 4.9 – 13.4)	8	5.1; 2.2 – 9.9)	19	11.7; 7.2 – 17.6)	0.112
Any illness	107	56.6; 49.2 – 63.8)	103	66.0; 58.0 – 73.4)	89	54.6; 46.6 – 62.4)	0.085

[a] Excluding varicella

Table 4.12 Childhood medications

	ICSI (n=189)		IVF (n=156)		Control (n=163)		*p*
	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	
Antibiotics	0	0.0 (0.0 – 1.6)	0	0.0 (0.0 – 1.6)	0	0.0 (0.0 – 1.8)	-
Asthma medication	28	14.8 (10.1 – 20.7)	28	14.8 (10.1 – 20.7)	17	10.4 (6.2 – 16.2)	0.325
Any medication	34	18.0 (12.8 – 24.2)	34	18.0 (12.8 – 24.2)	20	12.3 (7.7 – 18.3)	0.240

Table 4.13 Childhood surgery

	ICSI (n=189)		IVF (n=156)		Control (n=163)		*p*
	No.	% (95% CI)	No.	% (95% CI)	No.	% (95% CI)	
Any surgery	32	16.9 (11.9 – 23.1)	23	14.7 (9.6 – 21.3)	17	10.4 (6.2 – 16.2)	0.212
Circumcision	2	1.1 (0.1 – 3.8)	2	1.3 (0.2 – 4.6)	2	1.2 (0.2 – 4.4)	1.000
Other genitourinary surgery	8	4.2 (1.8 – 8.2)	4	2.6 (0.7 – 6.4)	3	1.8 (0.3 – 5.3)	0.433

Table 4.14 Growth

	ICSI (n=189)		IVF (n=156)		Control (n=163)		*p*
	Mean	**(95% CI)**	**Mean**	**(95% CI)**	**Mean**	**(95% CI)**	
Height – cms (unadjusted data)	110.5	(109.8 - 111.2)	109.6	(108.9 - 110.3)	109.6	(108.9 - 110.3)	0.207
Height centile (adjusted for age)	55.3	(51.1 - 59.5)	58.5	(53.8 - 63.2)	51.8	(47.2 - 56.4)	0.135
Weight – kgs (unadjusted data)	19.6	(19.1 - 20.1)	19.0	(18.6 - 19.4)	19.4	(18.9 - 19.9)	0.121
Weight centile (adjusted for age)	56.3	(52.1 - 60.5)	54.7	(50.0 - 59.4)	56.5	(52.1 - 60.9)	0.843
Head circumference – cms	52.1	(51.9 - 52.3)	52.3	(52.1 - 52.5)	52.0	(51.8 - 52.2)	0.072

Table 4.15 Audiometry

	ICSI (n=185)		IVF (n=154)		Control (n=159)		*p*	
	Left	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**
	No. (%) (95% CI)	**No. (%) (95% CI)**	**No. (%) (95% CI)**	**No. (%) (95% CI)**	**No. (%) (95% CI)**	**No. (%) (95% CI)**	0.792	0.700
Normal	155 (83.8) (77.7 – 88.8)	157 (84.9) (78.8 – 89.7)	134 (85.9) (79.4 – 91.0)	132 (84.6) (78.0 – 89.9)	135 (83.3) (76.7 – 88.7)	129 (79.6) (72.6 – 85.5)		
Abnormal	10 (5.4) (2.6 – 9.7)	10 (5.4) (2.6 – 9.7)	5 (3.2) (1.1 – 7.3)	7 (4.5) (1.8 – 9.0)	7 (4.3) (1.8 – 8.7)	11 (6.8) (3.4 – 11.8)		
No cooperation	14 (7.6) (4.2 – 12.4)	14 (7.6) (4.2 – 12.4)	13 (8.3) (4.5 – 13.8)	13 (8.3) (4.5 – 13.8)	11 (6.8) (3.4 – 11.8)	12 (7.4 (3.9 – 12.6)		
Difficult to assess	6 (3.2) (1.2 – 6.9)	4 (2.2) (0.6 – 5.4)	2 (1.3) (0.2 – 4.6)	2 (1.3) (0.2 – 4.6)	6 (3.7) (1.4 – 7.9)	7 (4.3) (1.8 – 8.7)		

There were 4 ICSI children, 2 IVF children and 4 control children who refused to participate in this assessment.

Table 4.16 Opthalmology

	ICSI (n=189)		IVF (n=156)		Control (n=163)		*p*
Abnormal results	**No.**	**(95% CI)**	**No.**	**(95% CI)**	**No.**	**(95% CI)**	
Cover test for strabismus	2	1.1 (0.1 – 3.8)	1	0.6 (0.0 – 3.5)	2	1.2 (0.2 – 4.4)	1.000
Motility test	0	0.0 (0.0 – 1.6)	0	0.0 (0.0 – 1.9)	0	0.0 (0.0 – 1.8)	--
Stereotactic vision	6	3.2 (1.2 – 6.9)	2	1.3 (0.1 – 4.6)	2	1.2 (0.2 – 4.4)	0.375
Visual acuity							
- Left	2	1.2 (0.1 – 4.1)	1	0.7 (0.0 – 3.6)	4	2.6 (0.7 – 6.6)	0.356
- Right	4	2.3 (0.6 – 5.8)	4	2.6 (0.7 – 6.6)	6	3.9 (1.5 – 8.3)	0.707

4.11 Results for Congenital Malformations (table 4.17)

The frequency of major malformations detected at birth was similar in all three groups. However, by later childhood, further major malformations had been detected in the ICSI and IVF group, but not in the control group (ICSI 3.2% vs IVF 2.6% vs control 0%; p=0.06). Overall there was a trend to higher major malformation rates in the ICSI and IVF group than the control group (ICSI 7.9% vs IVF 7.1% vs control 4.9%), but the numbers were too small to show significance (p=0.5).

Neonatal minor malformations were more common in the ICSI and IVF groups than the control group ($p<0.05$). The detection of further minor malformations in childhood was not significantly different between groups. Overall the minor malformation rate was higher in the ICSI and IVF group than the control group ($p<0.05$).

The overall malformation rates were higher in the ICSI and IVF groups than the control group ($p<0.05$). The malformations are listed in tables 4.18 – 4.21.

The data in table 4.17 gives information regarding the number of children with major and minor malformations in the neonatal and childhood periods. The same child may have more than one malformation, but will be counted only once in each of the minor and major groups. However, the same child may have another malformation detected between neonatal and childhood periods and would be counted on each occasion. For total number of malformations, the child would be counted once. Details of the number and type of malformations can be found on the subsequent tables.

4.12 Relationship between paternal sperm abnormalities and congenital malformations in ICSI children

Minor and major congenital malformations in ICSI children were compared with the paternal sperm abnormalities. Minor malformations were associated with asthenospermia ($p<0.05$) and post vasectomy, including non-reversal of vasectomy ($p<0.01$). Major abnormalities were not associated with any single sperm abnormality, but numbers were very small (see tables 4.22 and 4.23).

Table 4.17 Prevalence of malformations in UK children (N=508).

	ICSI (n=189)		IVF (n=156)		Control (n=163)		*p*
	No.	**% (95% CI)**	**No.**	**% (95% CI)**	**No.**	**% (95% CI)**	
Neonatal major malformations (a)	10	5.3 (2.6 – 9.5)	7	4.5 (1.8 – 9.0)	8	4.9 (2.1 – 9.4)	0.943
Neonatal minor malformations (b)	55	29.1 (22.7 – 36.1)	51	32.7 (25.4 – 40.7)	33	20.3 (14.4 – 27.2)	**<0.05**
Major malformations detected after neonatal period (up to 5 years) (c)	6	3.2 (1.2 – 6.8)	4	2.6 (0.7 – 6.4)	0	0.0 (0.0 – 1.8)	0.058
Minor malformations detected after neonatal period (up to 5 years) (d)	6	3.2 (1.2 – 6.8)	3	1.9 (0.4 – 5.5)	3	1.8 (0.3 – 5.3)	0.713
Total major malformation (e) (a OR c) *	15	7.9 (4.5 – 12.8)	11	7.1 (3.6 – 12.3)	8	4.9 (2.1 – 9.4)	0.514
Total minor malformation (f) (b OR d) *	55	29.1 (22.7 – 36.1)	52	33.3 (26.0 – 41.3)	34	20.9 (14.9 – 27.9)	**<0.05**
Overall malformations (e OR f) *	65	34.4 (27.7 – 41.6)	58	37.2 (29.6 – 45.3)	39	23.9 (17.6 – 31.2)	**<0.05**

*** These figures do not necessarily equal the sum of their components as some children will have had both types of malformation. Some children may have had malformations picked up in the neonatal period *and* in childhood.**

This table represents the number of individual children with malformations, but not the total number of malformations. Some children may have more than one malformation. Information about the total number of malformations can be seen in tables 4.18-4.21

Table 4.18 List of neonatal malformations for ICSI children

	Frequency
MAJOR	**10**
Congenital cataract	1
Duplication of ureter	1
Hydrocoele	1
Hypospadias	1
Meckel's diverticulum	1
Nasolacrimal ductus stenosis, unilateral	1
Other specified congenital malformations of male genital organs (scrotal fusion)	1
Peutz-Jeughers syndrome	1
Undescended testicle, unilateral	2
MINOR	**60**
Accessory nipple	1
Accessory skin tags	1
Congenital deformity of hand	2
Congenital dislocation of hip, bilateral	1
Congenital dislocation of hip, unilateral	1
Congenital malformations of the urinary system	1
Congenital morphological disturbances of hair, not elsewhere	1
Fused toes	1
Hernia inguinal	1
Macrocephaly	1
Mongolian blue spot	1
Other congenital deformities of hip	1
Other congenital malformations of abdominal wall (umbilical hernia)	4
Other congenital malformations of ear	5
Other congenital malformations of eyelid	1
Other congenital malformations of tongue	1
Other specified congenital malformations of ear	2
Other specified congenital malformations of eye	1
Other specified congenital malformations of skin	7
Patent ductus arteriosus	1
Preauricular sinus and cyst	1
Sinus, fistula and cyst of branchial cleft	1
Strawberry naevus	17
Talipes calcaneovarus	1
Talipes equinovarus	2
Ventricular septal defect	2
Webbed toes	1

Footnote
Major malformations are defined as "an abnormality causing impairment or requiring surgical intervention". Some malformation types can therefore be classified as major *or* minor for different individuals depending on the severity.

Table 4.19 List of neonatal malformations for IVF children

	Frequency
MAJOR	**7**
Congenital ptosis, blepharophimosis-ptosis syndrome	**1**
Hypospadias	2
Sinus, fistula and cyst of branchial cleft	**1**
Talipes equinovarus	1
Undescended testicle, unilateral	**2**
MINOR	**53**
Accessory skin tags	1
Hernia inguinal	**1**
Mongolian blue spot	1
Other congenital malformations of abdominal wall (umbilical hernia)	**4**
Other congenital malformations of ear	1
Other congenital malformations of eyelid	**1**
Other congenital malformations of face and neck	2
Other congenital malformations of kidney	**1**
Other congenital malformations of skull and face bones	1
Other congenital malformations of tongue, mouth and pharynx	**1**
Other misshapen ear	1
Other specified congenital malformations of ear	**1**
Other specified congenital malformations of eye	1
Other specified congenital malformations of skin	**16**
Strawberry naevus	18
Talipes equinovarus	**2**

Table 4.20 List of neonatal malformations for control children

	Frequency
MAJOR	**11**
Accessory finger	1
Adhesions labia minora	1
Cataract	1
Coarctation of aorta	1
Duplication of intestine	1
Dysplastic hip, bilateral	1
Hydrocoele	1
Other specified congenital non-neoplastic naevus	1
Portwine stain	1
Talipes Equinovarus, bilateral	1
Tetralogy of fallot	1
MINOR	**36**
Absence and agenesis of lacrimal apparatus	1
Congenital deformity of hand	1
Congenital malformation of eye, unspecified	1
Hernia inguinal, bilateral	1
Mongolian blue spot	1
Other congenital malformations of abdominal wall	2
Other congenital malformations of male genital organs	1
Other congenital malformations of testis and scrotum	2
Other congenital malformations of ureter	1
Other specified congenital malformations of skin	9
Strawberry naevus	13
Talipes equinovarus	1
Unstable hip, bilateral	2

Table 4.21 List of malformations in childhood

ICSI	**Frequency**
Major	**7**
Congenital deformities of feet	**1**
Congenital pyloric stenosis	1
Hydrocoele	**2**
Multiple exostoses	1
Strabismus	**1**
Undescended testicle, unilateral	1
Minor	**6**
Hernia inguinal, bilateral	1
Hernia inguinal, unilateral	**1**
Other specified congenital malformations of ear	1
Other specified congenital malformations of skin	**1**
Plagiocephaly	1
Strawberry naevus	**1**
IVF	
Major	**4**
Undescended testicle, unilateral	**2**
Cervical cyst	1
Labial cyst	**1**
Minor	**7**
Hernia inguinal	**1**
Other specified congenital malformations of eye	1
Other specified congenital malformations of skin	**1**
Plagiocephaly	1
Strawberry naevus	**1**
Undescended testicle, unilateral	2
CONTROL	
Major	**0**
No malformations	
Minor	**3**
Congenital malformation of eye, unspecified	1
Other congenital malformations of ribs	**1**
Other specified congenital malformations of skin	1

Table 4.22 Paternal sperm abnormalities and minor congenital malformations in the ICSI conceived child.

	No (%) malformations without sperm abnormality	**No (%) malformations with sperm abnormality**	***p***
Asthenozoospermia	41/119 (34%)	14/70 (20%)	**<0.05**
Oligozoospermia	25/97 (26%)	30/92 (33%)	0.30
Teratozoospermia	40/136 (29%)	15/53 (28%)	0.88
Failed IVF	47/165 (28%)	8/24 (33%)	0.63
Antisperm Ab's	50/174 (29%)	5/15 (33%)	0.71
Post vasectomy	48/179 (27%)	7/10 (70%)	**<0.01**
Post vasectomy reversal	50/180 (28%)	5/9 (56%)	0.12
Congenital absence of vas deferens	54/184 (29%)	1/5 (20%)	1.00
Other surgical obstruction	53/183 (29%)	2/6 (33%)	1.00

Note: There were 55 children who had one or more minor congenital abnormalities (see table 4.17)

Table 4.23 Paternal sperm abnormalities and major congenital malformations in the ICSI conceived child.

	No (%) malformations without sperm abnormality	**No (%) malformations with sperm abnormality**	***p***
Asthenozoospermia	10/119 (8%)	5/70 (7%)	0.76
Oligozoospermia	8/97 (8%)	7/92 (8%)	0.87
Teratozoospermia	12/136 (9%)	3/53 (6%)	0.56
Failed IVF	15/165 (9%)	0/24 (0%)	1.00
Antisperm Ab's	14/174 (8%)	1/15 (7%)	1.00
Post vasectomy	14/179 (8%)	1/10 (10%)	0.57
Post vasectomy reversal	15/180 (8%)	0/9 (0%)	1.00
Congenital absence of vas deferens	15/184 (8%)	0/5 (0%)	1.00
Other surgical obstruction	14/183 (8%)	1/6 (17%)	0.40

Note: There were 15 children who had one or more major congenital abnormalities (see table 4.17)

4.13 Wechsler Preschool and Primary Scale of Intelligence-WPPSI (Weschler 1990)

Data was available for 499 children for the WPPSI assessment. The remaining 11 children did not perform the assessment due to lack of cooperation and could not be assessed.

The data collected indicated that there was little difference between the groups (table 4.24). ICSI children had a slightly higher score for performance and full IQ. All groups performed better in the performance IQ compared with the verbal IQ. The mean results for all subscales in each conception group were greater than the child population mean of 100 which was calculated when the test was standardised in 1990.

4.14 McCarthy Motor Scales of Children's Abilities (McCarthy 1972)

There were 500 participating children in this assessment. For each conception group the children scored just above the population mean of 50 expected for the assessment (table 4.25). No abnormalities of motor skills could therefore be demonstrated in any group and no differences between conception groups could be demonstrated.

Table 4.24: WPPSI subscale results

	No.	Mean	SD	95% CI for mean	Significance between groups
Performance IQ					0.143
ICSI	188	107.83	12.51	106.03-109.63	
IVF	152	105.18	13.54	103.01-107.35	
Control	159	105.87	12.91	103.85-107.90	
Verbal IQ					0.730
ICSI	187	103.03	11.79	101.33-104.73	
IVF	152	103.85	10.20	102.21-105.48	
Control	159	102.86	13.17	100.79-104.92	
Full IQ					0.446
ICSI	187	106.26	11.62	104.58-107.93	
IVF	152	105.26	10.13	103.63-106.88	
Control	159	104.6	14.57	102.31-106.88	
Verbal-performance IQ					0.123
ICSI	187	-4.91	14.06	-6.94- -2.89	
IVF	152	-1.99	11.63	-3.86- -0.13	
Control	159	-3.31	13.33	-5.40- -1.22	

Children unable to cooperate with this assessment: 2 ICSI, 4 IVF and 4 controls

Table 4.25: McCarthy motor scales

	No.	Mean	SD	95%CI for mean	Significance between groups
Motor scale index					0.804
ICSI	187	50.73	8.51	49.51-51.96	
IVF	153	50.17	7.38	48.99-51.35	
Control	160	50.50	7.38	49.35-51.65	

Children unable to cooperate with this assessment: 2 ICSI, 3 IVF and 3 controls

4.15 Discussion of physical and neurodevelopmental results

4.15.1 Matching and Sociodemographic details of study families

Maternal and paternal ages were higher in the assisted reproductive therapy groups than the control parents. This is perhaps unsurprising as many parents who seek assistance to conceive will have had a period of unsuccessful attempts to conceive naturally before treatment will be considered. It is also well recognized that older mothers have reduced fertility and may be more likely to require assistance to conceive (Lansac 1995).

The majority of ICSI and IVF parents were from a social class higher than social class 3. This finding has been noted before (Bowen *et al.* 1998; Sutcliffe *et al.* 2001) – although the ICSI children in the Sutcliffe paper were mainly from the same cohort). The social class of the parents of control groups in this study was well matched. There was no difference between groups for social class of mothers or fathers. Control group mothers were more likely to have a university entry level education ($p<0.01$). This may be in part an effect of the rigorous matching for social class.

Mothers of ICSI/IVF children were more likely to be married than control mothers. Couples are not usually offered investigations for infertility unless they have had at least one year of failure to conceive naturally. Parents who are subsequently investigated and conceive after ICSI/ IVF treatment may therefore be more likely to be in a stable relationship compared with parents who conceived naturally.

Alcohol consumption and smoking was similar between maternal groups, which may also reflect the similar social class between groups. However, there were differences in the paternal groups, with control fathers more likely to smoke and ICSI/IVF fathers more likely to drink alcohol. Smoking has been demonstrated to reduce fertility (Hassan and Killick 2004) and it is possible that ICSI/IVF fathers may therefore have made a lifestyle decision not to smoke.

There was no difference between the three groups for numbers of mothers who had had at least one other pregnancy. Perhaps unsurprisingly, control mothers were more

likely to have other children, reflecting infertility and the high early pregnancy rate and loss after ICSI/IVF.

4.16 Physical outcomes of study

4.16.1 Perinatal outcomes

ICSI and IVF mothers reported more illnesses and complications during pregnancy. This increase was mainly accounted for by ovarian hyperstimulation syndrome, bleeding and placenta praevia. Ovarian hyperstimulation syndrome is recognized to be a complication of the ART procedures to stimulate egg development (Olivennes 2003). The incidence of this syndrome in ICSI/IVF mothers cannot therefore be compared with the naturally conceived group of mothers.

Bleeding and placenta praevia were also most common in the assisted reproduction groups. This is perhaps unsurprising as placenta praevia is a recognized complication of the embryo implantation process (Tan *et al.* 1992; Verlaenen *et al.* 1995) and is itself associated with an increased incidence of bleeding (Love and Wallace 1996). Previous studies have also found an increase in vaginal bleeding and hypertension (MRC 1990; Tan *et al.* 1992), but no increase in reporting of hypertension was found in this study. The use of medication was also highest in the IVF/ICSI groups, but differences were accounted for by the use of progesterone as part of the IVF treatment.

Although the numbers of mothers that were current drinkers was similar between groups, ICSI and IVF mothers were less likely to drink alcohol in pregnancy than control group mothers. This may reflect the desire to create the optimal environment for a precious pregnancy.

IVF mothers were most likely to work and control mothers were least likely. This is perhaps surprising, given that the control group of mothers had a higher educational level. Control mothers were less likely to be married which may influence their decision to work. However, it may be the case that ICSI/IVF mothers were more career minded and, in view of their increased age, they may have achieved higher levels of success at work which they would be reluctant to give up. It is also possible

that mothers who have had more interest in their careers have deferred motherhood and have hence found their fertility to be reduced and require assistance to conceive.

4.16.2 Neonatal outcomes

The ICSI children were more likely to be born after an emergency caesarian section and control children were most likely to be born by a non-instrumental vaginal delivery. An increased rate of caesarian section after IVF has been documented (Dhont *et al.* 1999), but this increase in emergency section / dysfunctional labour may be secondary to the increased maternal age in these groups rather than the assisted reproductive process (Berkowitz *et al.* 1990; Cohen *et al.* 1980).

The ICSI group were least likely to need resuscitation at birth and ventilation (not significant). It is possible that there is a correlation between the increase in emergency caesarian section and decreased need for resuscitation in this group. Parents of ICSI children and the medical staff responsible for the pregnancy and delivery may be more anxious about the outcome and intervene in the labour at an earlier stage, proceeding to emergency section and possibly negating the need for later resuscitation.

Despite the reduced need for resuscitation in the ICSI group, the numbers of children admitted to the neonatal unit was similar between groups. There was no difference seen between groups for reasons for admission to the neonatal unit or types of neonatal illness. There was an increase in sticky eyes and cephalhaematoma in the IVF group, but the numbers were small. The IVF children were less likely to have been delivered vaginally and more likely to have been born by emergency caesarian section than control children and the increase in cephalhaematoma is therefore unlikely to be attributable to traumatic vaginal delivery. It is possible that the increase in cephalhaematoma relates to difficulties in the third stage of labour for which the child had to be delivered by caesarian section.

There was no significant difference between groups for number of children who remained on the neonatal unit for ≥7days. However, if the number of babies who remained on the neonatal unit for ≥7days was considered in terms of just children who were admitted to the unit in each group, ICSI babies were more likely to be

admitted for greater than 7 days. In view of the fact that there was no increase in neonatal illness or type of illness in the ICSI group, this may simply reflect an increase in parental or physician anxiety. These findings support a previous study that also found no difference in neonatal outcomes between IVF and naturally conceived children, but a longer stay on the neonatal unit for the IVF children (Leslie *et al.* 1998).

ICSI babies were born at the lowest mean gestational age for the three groups and control babies at the highest, but this difference was not significant ($p=0.058$). The birth weight followed the same trend, with the lowest mean birth weight in the ICSI babies and highest in the control group. This was not found to be significant and may simply reflect the gestational maturity. There were also no differences noted for birth length or head circumference. Previous studies have reported an increase in prematurity and lower birth weight in ICSI/IVF children. (MRC 1990; Petersen *et al.* 1995; Gissler *et al.* 1995; Leslie *et al.* 1998; Dhont *et al.* 1999; Wennerholm and Bergh 2000; Koivurova *et al.* 2002). Our study excluded children born at a gestation <32 weeks.

The responses from mothers in each group for intention to breast feed were similar. However, the IVF mothers were least likely to exclusively breast feed and control mothers were most likely ($p<0.001$). Of those mothers who chose to breastfeed, the length of exclusive breastfeeding was similar between groups. The decreased rate of breastfeeding of IVF mothers compared to mothers of naturally conceived children has been reported before (Leslie *et al.* 1998). The reason for the reduction in exclusive breast feeding in the ICSI/IVF groups could be due to difficulties establishing breast feeding after caesarian section (Dewey *et al.* 2003) or due to the separation of mother and baby if the baby is admitted to the neonatal unit. In addition, bottle feeding can enable a father to be more involved in the care of the baby which may be of increased importance for fathers of ICSI / IVF children.

4.16.3 General health

There was a slight increase in the reporting of childhood illness, with parents of IVF children reporting a higher overall incidence of childhood illness than other parents, in particular dermatological and gastrointestinal complaints but this trend was not

significant (p=0.085). It is possible that the differences seen were secondary to parental over-anxiety or over-reporting. This is supported by the finding that there was no increase in childhood medication in these groups. This could be viewed as objective evidence that the children have similar rates of illness.

The general physical examination revealed few differences between the groups. There was an increase in adenopathy in the control group of children. This group of children was more likely to have siblings and perhaps had a greater exposure to common childhood infections that may lead to adenopathy.

There have been reports of a possible increase in genito-urinary abnormalities in ICSI children (Anthony *et al.* 2002; Foresta and Ferlin 2001; Sutcliffe *et al.* 2001; Wennerholm *et al.* 2000), but our study found that although there was a trend towards an increase in genitourinary abnormalities in ICSI and IVF boys, this was not significant (p=0.067). There was also an increase in genito-urinary surgery in ICSI/IVF boys but the numbers involved were too small to detect a significant difference statistically (ICSI 4.2% vs IVF 2.6% vs control 1.8%; p=0.4).

4.16.4 Growth

There were no differences between groups for the height and weight centiles for the children at the age of assessment. These findings support a previous case-matched control study of children conceived after assisted reproduction and found that the childhood weight was no different between groups (Saunders *et al.* 1996). This is reassuring in view of the recent concerns relating to an increase in Beckwith-Wiedemann syndrome in IVF conceived children (DeBaun *et al.* 2003; Maher *et al.* 2003) and evidence of overgrowth in other mammals after assisted conception (Khosla *et al.* 2001; McEvoy *et al.* 1998).

4.17 Congenital malformations

There appeared to be an increase in congenital malformations in the ICSI/IVF groups. Major malformations not detected at birth were detected in the ICSI and IVF groups at the age of five, supporting evidence that neonatal malformation registers miss a proportion of significant abnormalities (Simpson 1996).

To make a robust statement about the incidence of congenital malformation in the assisted conception groups compared with naturally conceived children, a much larger study of children would be required. However, there is evidence from this study that there may be increased rates of malformation in the assisted conception groups. There did not appear to be a difference in incidence of malformations between the ICSI and IVF groups, which suggested that the underlying cause of the malformations may be related to the genetic nature of the subfertile parents, rather than the process of ART itself.

The incidence of congenital abnormalities was assessed in ICSI children in relation to their father's sperm parameters. No clear link could be found between major abnormalities and sperm parameters, but there was correlation between minor congenital abnormalities and paternal asthenospermia ($p<0.05$) and post vasectomy ($p<0.01$). The numbers analysed are too small (tables 4.22 and 4.23) to draw conclusions from this data.

4.18 Neurodevelopmental outcomes

4.18.1 WPPSI

There was no IQ difference between groups. The mean results for all of the WPPSI subscales in each conception group were greater than the child population mean of 100 which was calculated when the test was standardised in 1990. This may represent a change in the population mean or it may represent an increased intelligence score in this group of study children due to social or educational advantage. The majority of children in all three conception groups had parents from social class 3 or higher and with generally good educational backgrounds.

4.18.2 McCarthy motor scales

There was no difference seen between children from each conception group for development of motor skills. Each group scored around the population mean of 50.

4.19 Strengths of the study

4.19.1 Sample size

The sample size was calculated to be sufficient to answer most of the study questions. ICSI was developed in 1992 and is a relatively new technique. Obtaining a larger cohort of ICSI children within the UK would not be possible using this age range. Therefore, questions that needed larger numbers could only be answered by an international collaboration. The data has therefore contributed to a European Collaboration, which is reported separately.

4.19.2 Comparison groups

Two groups were chosen, a naturally conceived control group and a conventional IVF comparison group. The control group provided a further standard of "normal" by which to compare the ICSI group. By comparing the control group to ICSI and IVF groups, it was possible to differentiate factors that were due to fertility treatments in general and those that were due to ICSI per se.

4.19.3 Outcome measures

The developmental assessment (WPPSI-R^{UK}) used in this study was standardised for the UK population of children and the outcomes are reproducible. The naturally conceived group provided further internal norms and the presence of an IVF comparison group allowed more detailed conclusions about assessment outcomes.

Conventional IVF techniques have been used since 1978, but there have been few assessments of developmental and physical outcomes. This study is therefore important in establishing the outcomes of conventional IVF children as well as ICSI.

4.19.4 Single and blinded psychologist

The blinding of the psychologist to mode of conception reduced observer bias. In addition all the children were assessed by the same psychologist (see chapter 3), providing consistent results and negating inter-observer bias.

4.19.5 Personal contact and advice lines

Many children in this study had been assessed at 18 months by Dr Sutcliffe. These families were kept up to date about the follow up studies and had been given the opportunity to contact those involved in the study for advice in the future. This service was extended to all new recruits involved in this follow up study. The continued contact with families allowed the paediatrician and psychologist to motivate families to participate and to complete all aspects of the study, including lengthy questionnaires.

4.20 Weaknesses of the study

4.20.1 Recruitment

The recruitment of IVF and ICSI children was limited by UK law. The Human Fertilisation and Embryology Act 1990 does not allow the HFEA or fertility clinics to disclose information about any individual who may have been conceived after assisted conception. Therefore any study in the UK must recruit using an "opt-in" method. We asked clinics to send letters of invitation to families on our behalf, but we only become aware of these families if they chose to respond to us and disclose their details. We were therefore unable to obtain data on those families that did not respond, and this could be a possible source of bias within our study.

4.20.2 Missing cases

An attempt was made to collect information on all families that had been recruited, but did not attend. This information was recorded in order to consider any possible source of bias. However, in a proportion of cases, families were either uncontactable due to a change of address or the family declined to participate. If the family declined to participate, they were asked if they were prepared to give a reason – several families did not wish to do so.

4.20.3 Cytogenetics

There is evidence that ICSI children have an increased risk of cytogenetic abnormalities (see chapter 1). It would therefore have been interesting to have obtained the cytogenetic profile of all the children in the study to ascertain the frequency and types of these abnormalities. This was not performed for two main

reasons. Firstly, the families and children would be less likely to participate in the study (many families specifically checked that blood tests would not be required).

Secondly, there are ethical issues regarding the genetic testing of children. The human genetics societies in Europe and the UK state that screening should clearly benefit the individual and where there is a possibility that some children may have effects from a genetic abnormality, it is best policy to wait until there is "something wrong". Overall, it is best to allow informed choice where possible and to wait until a child is old enough to provide consent

4.20.4 Sample size and congenital abnormalities

Although the sample size was adequate to obtain reliable outcomes for most of the outcomes measured, congenital abnormalities require much larger numbers than any UK study could provide. This data contributed to a larger European study that was more powerful.

A further difficulty in assessing the incidence of congenital abnormalities in children conceived after ART is that there is no clear denominator for the actual numbers of children conceived through the ART processes. The Human Fertility and Embryology Authority has some data, but many cases are missing. There is little or no information about spontaneous or therapeutic abortions following conception with ART. It is therefore uncertain if there are unidentified problems and that the study children are a "selected" surviving population. This may not be relevant when assessing the outcomes of ICSI and IVF children, but the study of these children may not give answers about the safety of ICSI and IVF per se.

4.20.5 Observer Bias

All children were assessed by a psychologist and paediatrician. Although the psychologist was blinded to conception type, the paediatrician was not. This was for practical administrative reasons. The paediatrician was involved in the recruitment of children into the study and arranged the appointments. Although the paediatrician performed the examination of children in a standardised manner it would have been more ideal if they had also been blinded to conception type. However, the lack of

blinding did allow the paediatrician to give advice to parents who had specific concerns about their child's health and the nature of their conception.

Chapter 4

KEY POINTS

- **ICSI babies were born at an earlier gestation than other groups, control group babies were born at a later gestation than other groups**
- **Birth weights were similar between conception groups**
- **Neonatal outcomes (retrospectively reported) were similar between groups**
- **IVF mothers were least likely to exclusively breastfeed, control mothers most likely**
- **Similar childhood hospital admission rates between groups**
- **ICSI and IVF children had greater incidence of genitourinary abnormalities in boys**
- **IVF and ICSI children had higher rates of childhood illnesses as reported by parents**
- **Medication intake between groups of children were similar**
- **No differences in growth at 5 years between groups**
- **More minor malformation were noted in ICSI and IVF children as neonates**
- **More major malformations were noted in ICSI and IVF children after neonatal period**
- **Overall minor and total malformations rates were highest in IVF and ICSI groups**
- **Childhood IQ scores were similar between groups**
- **Childhood motor development skills were similar between groups**

CHAPTER 5: CHILD AND FAMILY DEVELOPMENT STUDY

- RESULTS AND DISCUSSION OF CHILD AND FAMILY RELATIONSHIPS

5.1 Parental questionnaires to assess family functioning

5.1.1 Response rates

Parents were given the questionnaires and a reply-paid envelope at the child's assessment. Those parents who did not send back questionnaires were mailed a second copy. The overall response rates for the mother's questionnaires were between 50% and 63% (table 5.1). The results were similar between conception groups for mothers with the exception of the dyadic adjustment scales which had a lower response rate from the control group mothers.

There was a generally lower response rate of 45% to 55% from fathers, with the lowest response rate for all questionnaires from fathers of control group children.

5.1.2 Analysis

Univariate analyses were conducted, using ANOVA in SPSS 11.5 for Windows, to determine differences between the children conceived using ICSI, IVF and the naturally conceived Controls.

Table 5.1 Questionnaire response rates

Questionnaire	ICSI response rate 189 children	IVF response rate 158 children	Control response rate 163 children
MOTHER			
Child behaviour checklist (CBCL)	122 (65%)	94 (59%)	99 (61%)
Carey's temperament scale	113 (60%)	88 (56%)	93 (57%)
Parenting stress index (PSI)	118 (62%)	92 (58%)	94 (58%)
Parental acceptance and rejection (PARQ)	122 (65%)	94 (59%)	98 (60%)
Greenberger scales of work and parenting commitment*	118 (62%)	93 (59%)	92 (56%)
Dyadic adjustment scale (DAS)	112 (59%)	90 (57%)	81 (50%)
General health questionnaire (GHQ)	122 (65%)	96 (61%)	97 (60%)
FATHER			
Child behaviour checklist (CBCL)	104 (55%)	85 (54%)	74 (45%)
Carey's temperament scale	102 (54%)	77 (49%)	76 (47%)
Parenting stress index (PSI)	100 (53%)	83 (53%)	78 (48%)
Parental acceptance and rejection (PARQ)	102 (54%)	84 (53%)	79 (48%)
Greenberger scales of work and parenting commitment*	103 (53%)	84 (53%)	73 (45%)
Dyadic adjustment scale (DAS)	102 (54%)	82 (52%)	76 (47%)
General health questionnaire (GHQ)	105 (56%)	86 (54%)	80 (49%)

*This is a combined score for questionnaires on commitment to work and parenting. In cases where the response rate differed for each questionnaire, the best response is given in this table. The separate response rates can be seen in table 5.8.

5.2 Child Behaviour Checklist (CBCL)

Maternal reports of child behavioural psychopathology are similar between groups (table 5.2). However, the paternal reports differed - fathers of control children reported higher externalising behaviours for their child, such as aggression and temper tantrums ($p<0.05$) and higher total problem behaviour scores ($p=0.07$ - NS) than the fathers of IVF and ICSI children.

5.3 Carey Temperament Scales

Mothers of ICSI children reported higher (more difficult) levels of rhythmicity and intensity (tables 5.3 and 5.4). The differences between groups for rhythmicity was significant ($p<0.05$) with the greatest difference seen between the ICSI and IVF children (ICSI 4.2 vs IVF 4.0 vs control 4.1)For intensity, ICSI children were reported to have the highest scores, with control children scoring lowest ($p<0.05$). Other variables did not demonstrate differences between groups.

5.4 Parenting Stress Index (PSI)

There was a trend for ICSI mothers to report fewer symptoms of parental distress, dysfunctional parent-child interactions and difficult child behavioural than control mothers (tables 5.5 and 5.6). The total parenting stress index scores of the three groups of mothers were significant $p<0.05$, with the total PSI score for the ICSI mothers significantly lower than control mothers (ICSI 68.9 vs IVF 71.6 vs control 75.0; $p<0.05$) ie ICSI mothers described less manifestations of stress. There were no differences between fathers.

Table 5.2 Child behaviour checklist results

	No.	Mean	SD	95% CI for mean	*p*
MOTHER					
Internalising score					0.543
ICSI	122	47.06	8.79	45.48-48.63	
IVF	94	47.86	11.67	45.47-50.25	
Control	99	48.59	10.50	46.49-50.68	
Externalising score					0.174
ICSI	122	48.27	7.14	46.99-49.55	
IVF	94	47.97	9.64	45.99-49.94	
Control	99	50.11	9.61	48.20-52.03	
Social competence					0.713
ICSI	40	30.00	6.22	28.01-31.99	
IVF	0	0	0	0	
Control	32	29.34	8.86	26.15-32.54	
Total score					0.344
ICSI	122	49.62	8.71	48.06-51.18	
IVF	94	49.83	10.58	47.66-52.00	
Control	99	51.44	10.29	49.39-53.50	
FATHER					
Internalising score					0,1
ICSI	104	45.7	8.3	44.04-47.30	
IVF	85	46.0	11.2	43.60-48.45	
Control	74	48.8	10.4	46.37-51.20	
Externalising score					**<0.05**
ICSI	104	46.9	7.4	45.49-48.35	
IVF	85	45.8	8.8	43.90-47.68	
Control	74	49.5	8.8	47.47-51.53	
Social competence					0.8
ICSI	36	28.50	6.44	26.32-30.68	
IVF	0	0	0	0	
Control	23	28.00	8.65	24.26-31.74	
Total score					0.07
ICSI	104	48.4	8.4	46.76-50.03	
IVF	85	48.1	11.2	46.30-49.84	
Control	74	51.0	0.95	48.75-53.17	

Table 5.3 Carey Temperament Subscales

	ICSI		IVF		Controls		*p*
	Mean (1-7)	SD	Mean (1-7)	SD	Mean (1-7)	SD	
MOTHER							
Activity	4.7	0.61	4.6	0.65	4.7	0.60	0.09
Rhythmicity	4.2	0.68	4.0	0.59	4.1	0.62	**<0.05**
Intensity	4.0	0.61	3.9	0.61	3.8	0.52	**<0.05**
Distractability	4.1	0.48	4.1	0.53	4.2	0.61	0.94
Adaptability	3.8	0.56	3.8	0.57	4.0	0.59	0.07
Approach	3.9	0.58	3.9	0.61	4.0	0.58	0.69
Mood	5.1	0.60	5.0	0.65	5.0	0.62	0.47
Persistence	4.5	0.64	4.6	0.73	4.6	0.56	0.48
Threshold	3.7	0.65	3.7	0.67	3.7	0.60	0.82
FATHER							
Activity	4.7	0.55	4.8	0.58	4.8	0.59	0.64
Rhythmicity	4.3	0.69	4.12	0.56	4.2	0.63	0.35
Intensity	4.1	0.55	4.0	0.62	4.0	0.61	0.54
Distractability	4.2	0.57	4.1	0.53	4.2	0.63	0.31
Adaptability	3.8	0.60	4.0	0.60	4.0	0.61	0.08
Approach	4.0	0.53	3.9	0.53	4.0	0.61	0.35
Mood	4.8	0.63	4.7	0.64	5.0	0.64	0.06
Persistence	4.6	0.61	4.5	0.58	4.6	0.67	0.40
Threshold	3.9	0.66	3.8	0.81	3.7	0.69	0.35

Table 5.4 Carey Temperament Scales: total scores

	No.	Mean (0-84)	SD	95%CI for mean	*p*
MOTHER total					0.11
ICSI	113	38.2	3.03	37.5-38.7	
IVF	88	37.3	3.21	36.6-38.0	
Control	93	37.8	2.59	37.3-38.4	
FATHER total					0.22
ICSI	102	38.4	2.83	37.8-38.9	
IVF	77	37.8	2.76	37.2-38.4	
Control	76	38.6	3.36	37.8-39.4	

Table 5.5 Parenting stress index subscales

	ICSI		IVF		Controls	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
MOTHER						
Parental distress (12-60)	24.5	6.0	24.9	8.2	26.5	8.0
Parent-child dysfunctional interaction (12-60)	17.9	5.4	18.5	5.4	19.7	7.0
Difficult child behaviour (12-60)	26.9	8.1	28.2	8.3	28.6	9.2
FATHER						
Parental distress (12-60)	24.2	8.7	24.8	7.8	25.5	7.5
Parent-child dysfunctional interaction (12-60)	19.6	7.9	18.6	5.2	19.2	5.3
Difficult child behaviour (12-60)	26.3	7.7	26.9	8.1	27.4	7.4

Table 5.6 Parenting stress index: total results

	No.	**Mean** (0-84)	**SD**	**95%CI for mean**	***p***
MOTHER total					**<0.05**
ICSI	118	68.9	14.4	66.5-71.7	
IVF	92	71.6	16.8	68.2-75.1	
Control	94	75.0	18.9	71.1-78.8	
FATHER total					0.69
ICSI	100	69.9	21.1	65.7-74.1	
IVF	83	68.9	21.4	64.3-73.6	
Control	78	71.6	15.6	68.1-75.1	

5.5 Parental Acceptance and Rejection Questionnaire (PARQ)

Parents in all groups scored highly for reported parental warmth towards their child with no differences between groups seen (table 5.7). However, differences were seen between groups for maternal and paternal reports of hostility and rejection. ICSI mothers and fathers were less likely to report feelings of aggression/hostility than parents of controls. For both parent groups differences between the three conception groups were significant for hostility (mothers $p<0.01$, fathers $p<0.05$). The reports of feelings of rejection were also less in ICSI mothers but differences between the three conception groups were not significant ($p=0.06$). The total scores for mothers was not significant between groups, reflecting no difference in negative feelings towards their children ($p=0.342$).

Differences between fathers for feelings of rejection towards their child were significant with fathers of control children were more likely to report feelings of rejection than ICSI or IVF fathers (ICSI 16.27 vs IVF 14.94 vs control 14.53; $p<0.05$). The total scores for fathers, reflecting negative feelings towards their children, although not high overall, was different between the groups ($p<0.05$).

These findings do not support the hypothesis that parents of ICSI children will have more difficult family relationships (see discussion 5.10).

Table 5.7 PARQ subscales

		No.	Mean	SD	95%CI for mean	*p*
MOTHER						
Warmth	(20-80)					0.157
ICSI		122	77.2	3.4	76.47-77.76	
IVF		94	76.3	3.6	75.24-77.02	
Control		98	76.8	3.4	76.15-77.50	
Hostility	(15-60)					**<0.01**
ICSI		122	26.34	5.30	25.39-27.29	
IVF		94	26.71	6.25	25.43-27.99	
Control		98	28.65	6.12	27.43-27.99	
Neglect	(15-60)					0.132
ICSI		122	20.75	3.25	20.16-21.33	
IVF		94	21.78	4.02	20.95-22.60	
Control		98	21.34	4.10	20.51-22.16	
Rejection	(10-40)					0.06
ICSI		122	14.74	3.29	14.15-15.33	
IVF		94	15.55	3.71	14.79-16.31	
Control		98	15.83	3.69	15.09-16.57	
Total score	(60-240)					0.342
ICSI		122	85.4	13.6	83.31-88.19	
IVF		94	87.4	13.5	84.81-90.45	
Control		98	87.2	15.0	85.61-91.12	
FATHER						
Warmth	(20-80)					0.449
ICSI		102	74.8	5.1	73.74-75.76	
IVF		84	74.4	5.3	73.23-75.53	
Control		79	73.7	5.7	72.47-75.00	
Hostility	(15-60)					**<0.05**
ICSI		102	25.56	5.85	24.41-26.71	
IVF		84	25.99	6.75	24.52-27.45	
Control		79	28.35	7.13	26.76-29.95	
Neglect	(15-60)					0.720
ICSI		102	22.79	4.59	21.89-23.70	
IVF		84	22.88	5.30	21.73-24.03	
Control		79	23.37	5.03	22.24-24.49	
Rejection	(10-40)					**<0.05**
ICSI		102	14.53	4.04	13.74-15.32	
IVF		84	14.94	3.89	14.10-15.78	
Control		79	16.27	4.27	15.31-17.22	
Total score	(60-240)					**<0.05**
ICSI		102	88.3	14.6	85.46-91.20	
IVF		84	89.4	16.2	85.91-92.95	
Control		79	94.3	17.5	90.33-98.18	

5.6 Greenberger Scales of Work and Parenting Commitment

For mothers, there was a trend towards lower commitment to work in the ICSI and IVF group than the control group but this was not significant ($p=0.5$) (table 5.8). However, mothers of ICSI children had a higher commitment to their role as a parent than mothers in the other two groups ($p<0.001$).

There was a trend to differences between the groups for father's commitment to work ($p=0.058$) with fathers of control children scoring higher. Differences between groups for father's commitment to parenting were not significant ($p=0.5$), although fathers of ICSI children had a higher mean score than the other groups.

5.7 Dyadic Adjustment Scale (DAS) results

There were no differences seen between conception groups for any of the parental DAS subscores measured (table 5.9). A total dyadic adjustment score for parents therefore also showed no significant differences between groups (table 5.10).

5.8 General Health Questionnaire (GHQ-28) results

There were significant differences seen between groups for maternal severe depression ($p<0.01$). Mothers of control children reported higher levels of severe depression (table 5.11). Control mothers had more symptoms for severe depression when compared with ICSI or IVF mothers (ICSI 0.07 vs IVF 0.06 vs control 0.30; $p<0.01$). There was also a trend towards less anxiety and insomnia in the IVF and ICSI mothers compared to controls, but this did not reach significance.

There were no reported differences between fathers for severe depression. There was a trend for less symptoms of social dysfunction in the IVF and ICSI groups compared with controls but this did not reach significance.

Despite the differences in subscales reported above, the total scores for the GHQ for mothers and fathers were similar between groups and not significant (table 5.12).

Table 5.8 Greenberger subscales

	No.	Mean (17-102)	SD	95%CI for mean	*p*
MOTHER					
Work commitment					0.519
ICSI	99	50.77	12.31	48.31-53.22	
IVF	76	50.45	11.09	47.91-52.98	
Control	74	52.53	12.70	49.58-55.47	
Parenting commitment					**<0.001**
ICSI	118	76.05	8.04	74.58-77.52	
IVF	93	71.65	9.20	69.75-73.54	
Control	92	71.27	11.15	68.96-73.58	
FATHER					
Work commitment					0.058
ICSI	102	59.08	11.56	56.81-61.35	
IVF	82	56.56	13.19	53.66-59.46	
Control	75	61.31	12.69	58.39-64.23	
Parenting commitment					0.526
ICSI	103	71.67	9.53	69.81-73.53	
IVF	84	70.93	7.43	69.32-72.54	
Control	73	70.25	7.13	68.58-71.91	

Table 5.9 Dyadic adjustment scales (DAS) subscales

	ICSI		IVF		Controls	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
MOTHER						
Satisfaction (0-50)	33.5	5.3	33.9	5.4	32.5	5.6
Cohesion (0-24)	14.3	3.7	13.7	3.6	14.3	4.3
Consensus (0-65)	49.7	7.6	50.2	7.2	49.4	7.7
Expression of affection (0-12)	8.8	2.5	8.5	2.2	8.8	2.5
FATHER						
Satisfaction (0-50)	32.9	5.1	34.0	5.3	34.9	5.4
Cohesion (0-24)	14.7	3.4	14.7	3.8	14.8	4.5
Consensus (0-65)	49.4	6.7	48.7	7.5	48.6	6.9
Expression of affection (0-12)	8.7	2.2	8.4	2.0	8.4	2.2

Table 5.10 Total dyadic adjustment scores for parents (DAS)

	No.	**Mean** (0-151)	**SD**	**95%CI for mean**	***p***
MOTHER total					0.521
ICSI	112	105.65	16.57	102.55-108.75	
IVF	90	106.89	13.84	103.99-109.79	
Control	81	104.17	15.75	100.69-107.65	
FATHER total					0.540
ICSI	102	106.87	13.01	104.32-109.43	
IVF	82	106.40	14.47	103.22-109.58	
Control	76	104.62	14.08	101.40-107.84	

Table 5.11 GHQ subscales

	ICSI		IVF		Controls	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
MOTHER						
Somatic symptoms	1.08	1.8	0.93	1.6	1.00	1.7
Anxiety and insomnia	0.75	1.4	0.64	1.4	0.93	1.7
Social dysfunction	0.63	1.4	0.51	1.4	0.63	1.5
Severe depression *	**0.07**	0.33	**0.06**	0.35	**0.30**	0.90
FATHER						
Somatic symptoms	0.95	1.6	0.93	1.7	1.09	1.7
Anxiety and insomnia	0.70	1.4	0.69	1.5	0.84	1.5
Social dysfunction	0.39	1.0	0.45	0.9	0.63	1.5
Severe depression	0.15	0.77	0.15	0.65	0.15	0.51

*Maternal severe depression **p<0.01**

Table 5.12 General Health Questionnaire (GHQ-28): total scores

	No.	**Mean** (0-84)	**SD**	**95%CI for mean**	***p***
MOTHER total					0.344
ICSI	122	2.53	3.99	1.82-3.25	
IVF	96	2.09	3.58	1.37-2.82	
Control	97	2.92	4.12	2.09-3.75	
FATHER total					0.597
ICSI	105	2.19	3.52	1.51-2.87	
IVF	86	2.17	3.50	1.42-2.93	
Control	80	2.68	3.91	1.80-3.55	

5.9 Bene-Anthony family relations test

Of the 510 children studied, a total of 460 children performed the Bene-Anthony test. The main determinant for children not performing the assessment was the presence of one or more parent. If the parent was in earshot, the child may not be able to participate without bias.

Fathers of ICSI children had a significantly higher number of normal positive scores from their children than fathers of IVF or naturally conceived children (table 5.13). There did not appear to be any significance difference between groups for the children's positive or negative feelings towards their mothers or negative feelings towards fathers.

Table 5.13 Bene-Anthony family relations test

	No.	Mean	SD	95% CI for mean	*p*
Mothers positive score					0.826
Control	146	6.47	3.29	5.93-7.00	
ICSI	168	6.52	3.76	5.95-7.10	
IVF	146	6.28	3.64	5.69-6.88	
Mothers negative score					0.833
Control	146	2.32	2.52	1.90-2.73	
ICSI	168	2.33	2.93	1.89-2.78	
IVF	146	2.16	2.47	1.76-2.57	
Fathers positive score					**<0.01**
Control	146	3.64	2.89	3.17-4.12	
ICSI	168	4.71	3.63	4.15-5.26	
IVF	146	3.60	2.66	3.17-4.04	
Fathers negative score					0.611
Control	146	2.92	2.93	2.44-3.40	
ICSI	168	2.95	3.21	2.46-3.43	
IVF	146	3.25	3.33	2.70-3.79	

5.10 Discussion of family relationship data

5.10.1 Response rate to questionnaires

The response rate was as expected for this type of postal survey (Cummings *et al.* 2001; Boreham *et al.* 2003). No socio-demographic differences between non-responders and responders could be found. It is possible that parents with more stable relationships may reply whilst those with difficulties remain silent. Golombok has previously hypothesized that where questionnaires are used, it is possible that IVF families may wish to portray a more positive picture in order to "prove" that they are good parents (Golombok *et al.* 2001). Alternatively, the higher response rate from assisted conception families may be a courtesy effect, where parents of IVF / ICSI children were more willing to cooperate because of the medical assistance with their conception (van Balen *et al.* 1996).

Overall the fathers of each group had lower response rates. This may be in part due to fathers not always living in the same household as the child. In all cases, except one, the child was primarily living with the mother. In addition, fathers of control children may view the questionnaires as less important or relevant than fathers of children conceived after IVF or ICSI.

The lower response rate for the control fathers' questionnaires may in part be due to the marital status of the control group parents. There was a higher rate of separation and divorce in the control group. If a parent (usually the father) is not living with the child, they may be more reluctant to complete the questionnaire. In addition, parents that were separated or divorced were not asked to complete the dyadic adjustment scale.

5.10.2 Child socio-emotional behaviour

The parents of ICSI and IVF children in general reported fewer behavioural problem scores than parents of control children. In particular, fathers of ICSI and IVF children recorded significantly lower levels of externalising behaviours such as temper tantrums and aggression than fathers of control children. These findings support a previous teacher–rated study which found a reduction in child behavioural problems in IVF children compared with naturally conceived children (Hahn and DiPietro 2001). Sutcliffe et al also found that parents of ICSI children reported fewer

behavioural problems than naturally conceived controls when studied at 18 months (Sutcliffe *et al.* 2004). The cohort of ICSI children studied was the same as studied in this thesis, and thus these findings at 5 years of age are consistent with the findings of the study at 18 months.

However, parents of ICSI children reported some aspects of more difficult temperament when compared to reports from parents of controls. ICSI mothers reported higher levels of rhythmicity (of biological functions) and intensity than mothers of control children or IVF children, implying that the children have more difficult temperaments. Both parental groups reported decreased adaptability of ICSI children and fathers of ICSI and IVF children reported their children to be more negative in mood than fathers of control children. It is possible that parents of the assisted conception children have more intense relationships with their child and are more over-protective (Golombok *et al.* 2001; McWhinnie 1996) and this in turn leads to the differences in childhood temperament

5.10.3 Parental behaviour

ICSI and IVF mothers reported fewer symptoms of maternal severe depression than control mothers. There was also a trend towards lower anxiety and insomnia in IVF and ICSI mothers. ICSI mothers also reported fewer symptoms of parental distress using the parental stress index with an overall lower score for this questionnaire than control mothers. IVF mothers had an intermediate parenting stress index score. This confirms findings of previous studies which indicate that parenting stress is reduced in families of IVF children (Hahn 2001; Greenfeld *et al.* 1996; Golombok *et al.* 2001; Hahn and DiPietro 2001; van Balen 1996) There were also no differences between groups of fathers for general health or parental stress.

One possible reason for the reduction in maternal depression in the families of assisted conception children may be related to the level of satisfaction gained from the motherhood role. Mothers of ICSI children had a much higher commitment to their role as a parent than mothers in the other two groups. Colpin et al found that IVF mothers had stronger motivation scores for identity and motherhood (Colpin *et al.* 1998) and van Balen found that IVF mothers experienced more pleasure in their child and reported more parental competence than did the naturally fertile mothers

(van Balen 1996). Father's of ICSI children in this study had a trend towards a higher commitment to parenting than the other groups which may support a study that found that assisted reproduction fathers interacted more with their children and contributed more to parenting than fathers of naturally conceived children (Golombok *et al.* 1996).

A previous study found that mothers of IVF children reported working more for economic reasons than for professional development and the authors hypothesised that these mothers would rather stay at home with their children (Colpin *et al.* 1995) and may have a lower commitment to work. This study found a trend towards lower commitment to work in the ICSI and IVF group than the control group, but this was not significant. However, a difference was seen for father's commitment to work. IVF and ICSI fathers scored lowest, with fathers of control children having the highest commitment to work.

There were no differences seen between conception groups for any of the parental total dyadic adjustment score or any of its subscales indicating that the relationships between parents in all three groups does not differ confirming previous findings that parents in assisted reproduction families seemed to have stable marriages (Golombok *et al.* 2002).

5.10.4 Parent-child relationship

Levels of warmth towards children were similar between parents of the three conception groups. This contrasts a previous study which found increased levels of warmth in parents of IVF-conceived children (Golombok *et al.* 1996).

Parents of ICSI and IVF children were less likely to report feelings of aggression/hostility than parents of controls, despite reports that these children have more difficult temperaments. It is possible that these parents are currently suppressing their negative feelings in order to protect their child. However, if this is the case, the parent-child interactions may change when the child reaches adolescence and displays a different range of behaviours and temperaments whilst seeking more independence. Follow-up of these families could explore this further.

Mothers of ICSI children, together with fathers of ICSI and IVF children also reported less symptoms of rejection. These findings may be a reflection of the increased commitment to parenting, particularly for fathers. In the case of ICSI fathers, the lower levels of perceived rejection may relate to the higher number of normal positive Bene-Anthony scores from their children. This again supports the theory that these fathers of assisted conception children interact more with their children and contribute more to parenting (Golombok *et al.* 1996).

This family relationship study of ICSI, IVF and naturally conceived children finds no adverse effects on the family relationships of assisted conception families. The parents of ICSI and IVF children appear to be slightly more committed to parenting and the temperament differences of their children compared to naturally conceived control children may reflect a more intense parent-relationship. The parents of the assisted conception children had to go through a series of investigations and treatments in order to conceive and this could be argued to be a form of selection for "desire to become parents". Naturally conceived parents did not have this selection process and this may in part account for the differences seen for commitment towards parenting and reporting of the parent-child relationship. The outcome in adolescence and in the long term can only be assessed by future follow up studies.

Chapter 5

KEY POINTS

Child behaviour

- ICSI and IVF children were reported to have fewer behavioural problem scores, with less externalising behaviours such as temper tantrums and aggression
- ICSI children may have more difficult temperaments, with higher levels of rhythmicity and intensity and decreased adaptability compared with IVF or control children
- ICSI and IVF children may be more negative in mood than control children

Parental behaviour

- ICSI and IVF mothers reported less symptoms of severe depression than control mothers and ICSI mothers reported fewer symptoms of parental stress
- Mothers of ICSI children had a much higher commitment to their role as a parent than mothers in the other two group
- IVF and ICSI fathers had lower commitment to work than fathers of control children

Parent-child relationship

- Parents of ICSI and IVF children were less likely to report feelings of aggression/hostility than parents of controls, despite reports that these children have more difficult temperaments
- Mothers of ICSI children, together with fathers of ICSI and IVF children also reported less symptoms of rejection
- ICSI children reported more positive interaction with their fathers than other children

CHAPTER 6: PARENTAL ATTITUDE SURVEY

- INFORMING CHILDREN ABOUT THEIR MODE OF CONCEPTION

6.1 Background

There has been little study around the area of parental decisions to inform IVF-conceived children of their mode of conception. Studies to date are discussed in chapter 1 (1.10.3). Two studies found that over half the parents did intend to tell their child at some point (Colpin and Soenen 2002; Greenfeld *et al.* 1996). Despite this intention, several studies have found that the majority of parents with children under 10 years of age conceived using in-vitro fertilisation (IVF) had not revealed the method of conception (Colpin and Soenen 2002; Greenfeld *et al.* 1996; McWhinnie 1996; Olivennes *et al.* 1997; Braverman *et al.* 1998; Brewaeys *et al.* 1997).

Other studies investigating the issue of disclosure have focused mainly on donor insemination (DI) families (Golombok *et al.* 1996; Golombok *et al.* 2002). These studies may not be representative of IVF families per se. The use of donor eggs or sperm and the subsequent lack of genetic link to one or other parent may lead to differences in parental expectations of the reactions to disclosure by the child or extended family.

Studies of adults who were adopted as children, have shown that it is important that they are told of their adoption at an early age and provision of information about their genetic background helped in the development of a stable identity (Hoopes 1990). Thus, informing children about their mode of conception at an earlier age may result in a more favourable outcome in terms of identity and emotional issues.

This study examined parental attitudes towards informing their genetically related IVF/ICSI child of their mode of conception. It also examined the issue of the motives behind a parent's decision about whether or not they intend to tell their child. The study findings have been accepted for scientific publication (Peters *et al.* 2005).

6.2 Methods

This study included all parents of families involved in the child and family outcome study who had children conceived after ICSI or conventional IVF. The families were sent an explanatory letter and each parent was asked to complete a questionnaire with a request that they did not confer.

This questionnaire (appendix 6) was designed by the present author (Research paediatrician) and Xenya Chrysostomou (Research psychologist) with supervision from Dr Alastair Sutcliffe (Paediatrician) and Professor Jacqueline Barnes (Psychologist). A number of points were investigated:

(i) Whether parents had revealed their child's method of conception to others and if so, to whom.

(ii) Whether parents had discussed the method of conception with their child.

(iii) Whether parents had decided if they intended to tell their child how they were conceived.

(iv) If intending to tell, at what age did they wish to inform their child?

(v) If undecided whether to tell, what were their concerns?

(vi) If parents did not want to inform their child, why not?

(vii) Were parents able to find any literature, short films or any other material addressing the issue of telling children that they were conceived after assisted conception?

(viii) Did parents want literature to help them inform their child, and if so, what would be helpful to them?

For each question, a list of potential responses was provided. The parents could tick as many answers as were applicable.

6.2.1 Analysis

The association between factors and outcomes were tested using the chi-square test, whilst odds ratios and confidence intervals were calculated directly from relevant 2x2 contingency tables. T-tests were used to compare the means of continuous variables.

6.3 Results

6.3.1 Sample size

Questionnaires were returned by families of 181 children (response rate 51%), 145 (80%) with data from both parents, 31 (17%) with data from the mother only and 5 (3%) with data from the father only.

6.3.2 Revealing the child's conception method to family, friends and others

Most parents had told somebody about their child's method of conception and they were most likely to have confided in close friends and family (Table 6.1). More than half (56% mothers; 53% fathers) did not mind who knew while only 1% of mothers and 3% of fathers had told no-one.

Parental couples did not always agree in their response. For the 145 families with data from both parents, there were 15 (10%) families in which the father did not mind who knew but the mother did, and a further 15 (10%) families in which the mother did not mind who knew but the father did. In 3 families the father had told no-one but the mother had told close friends. There were no cases the mother had told no-one but the father had told someone.

6.3.3 Informing the child of their method of conception

Parental responses to the question of whether they had already told or had intention to tell their child how they were conceived are shown in Table 6.2 and the age at which they had told or intended to tell are shown in Figure 6.1. Of the 176 mothers in the study, 46 (26%) had told their child their method of conception, compared with 25/150 fathers (17%). Of these parents, 91% of mothers and 100% fathers also gave the age the child had been when told. The mean age for mothers was 3.7 years (SD 1.1 years, range 1-6). The mean age for fathers was 3.5 years (SD 1.3 years, range 1-5). For the 145 children where the data was available from both parents, 38 had been told about their method of conception. Of these children, 22 had been told by both parents, 3 had been told by father only and 13 by mother only.

Table 6.1 Frequency of responses to question 1, *"Who have you told about your child's methods of conception?"*

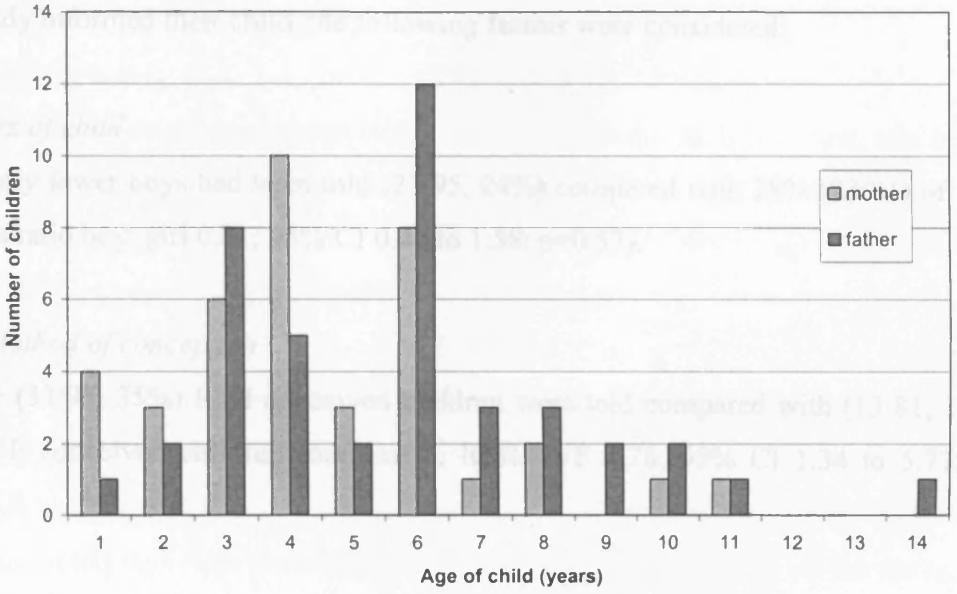
Who have you told?	***Mothers***		***Fathers***	
	n=176	(%)	*n=150*	(%)
My set of parents	149	(85%)	121	(81%)
Partner's set of parents	135	(77%)	127	(85%)
Both sets of parents	133	(76%)	117	(78%)
My/our other children §	15	(16%)	15	(19%)
Close friends	152	(86%)	116	(77%)
Other family members	144	(82%)	120	(80%)
Professionals	59	(34%)	46	(31%)
We do not mind who knows	99	(56%)	80	(53%)
No-one	2	(1%)	5	(3%)

§ Answers to this question are restricted to those parents who report having more than one child

Table 6.2 Intention of parents to inform child of their method of conception

Intention to inform child	***Mothers***		***Fathers***	
	n=176	(%)	*n=150*	(%)
Yes, already told child	46	26%	25	17%
Yes, at some point	102	58%	86	57%
Undecided	28	16%	31	21%
No, never	0	0%	6	4%
No response	0	0%	2	1%

Figure 6.1 Age at which parents intend to inform their children



6.3.4 Factors associated with parental disclosure to their child

Several factors were considered that may be related to a parent's decision to inform their child of their method of conception. Of the 46 mothers (out of 176) who had already informed their child, the following factors were considered:

(i) Sex of child

Slightly fewer boys had been told (23/95, 24%) compared with 28% (23/81) of girls (odds ratio boy: girl 0.81; 95% CI 0.41 to 1.58; p=0.53).

(ii) Method of conception

More (33/95, 35%) ICSI conceived children were told compared with (13/81, 16%) of IVF conceived children (odds ratio, ICSI: IVF 2.78; 95% CI 1.34 to 5.77; p< 0.01).

(iii) Presence of at least one sibling

More only children had been told (28/85, 33%) compared with (18/91, 20%) of those having one or more siblings (odds ratio, no siblings: at least one 1.99; 95% CI 1.00 to 3.96; p<0.05).

Of the 150 fathers, 25 reported that they had told their child. Assessing the possible influencing factors, no differences were found for sex of child (odds ratio, boy: girl 1.07; 95% CI 0.53 to 2.30, p=0.88), method of conception (odds ratio, ICSI: IVF 1.18; 95% CI 0.49 to 2.79, p = 0.71) or presence of at least one sibling (odds ratio, no siblings: at least one 1.34; 95% CI 0.63 to 2.61, p = 0.51).

(iv) Naturally Conceived Siblings vs IVF Siblings

The presence IVF or naturally conceived siblings was not associated with parental attitudes to disclosure. 35 out of 45 mothers (78%) who had at least one naturally conceived child said that they had or would tell their child about their method of conception. In contrast, 40 out of 46 mothers (87%) who had no naturally conceived children said that they had or would tell their child about their method of conception. This difference was not significant at the 5% level (OR=0.53; 95% CI 0.17 to 1.59). Thirty one out of 43 fathers (72%) who had at least one naturally conceived child said that they had or would tell their child about their method of conception. In

contrast, 30 out of 37 fathers (81%) who had no naturally conceived children said that they had or would tell their child about their method of conception. This difference was not significant at the 5% level (OR=0.60; 95% CI 0.21 to 1.74).

(v) Age of parent

Parental age was not associated with whether or not the child had been told by the mother about their method of conception. For maternal age, odds ratio per year increase was 0.94 (95% CI 0.87 to 1.02, p=0.13). For father's age, odds ratio per year increase was 0.98 (95% CI 0.90 to 1.05, p=0.52).

6.3.5 Relationship between informing the child and informing other adults

Telling others was associated with telling the child in question. Of the 99 mothers who did not mind who knew about their method of conception, 93 (94%) said they had or would tell their own child, compared with 71% (55/77) of the remaining mothers (OR=6.2; 95% CI 2.4 to 16.2). Similarly, of the 80 fathers who do not mind who knew, 71 (89%) said they had or would tell their own child, compared with 59% (40/68) of the remaining fathers (OR=5.5; 95% CI 2.4 to 12.9).

Only two mothers and five fathers had told no-one. It is therefore not possible to assess how likely these parents are to tell their own child relative to other parents.

6.3.6 Disclosure in the future

a) Age at which parents intend to disclose conception method to their child

Of the 102 mothers who said they intended to tell the child (Table 6.2), 42 (41%) specified an age (mean 8.6 years, s.d. 2.2). Of the 86 fathers who said they intended to tell their child (Table 6.2), 41 (48%) specified an age (mean 9.9 years, s.d. 2.7).

In the 21 families where both the mother and father said they intended to tell the child and both gave the age at which they intended to tell the child the correlation between the mother's and father's responses was 0.68. The age reported by the father was on average 0.76 years older than that reported by the mother (p=0.28; 95% CI – 0.7 years to 2.2 years).

b) Reasons given by parents who do not wish to disclose conception method to their child

No mother said that she would never tell her child but six fathers gave this response (Table 6.2). Only one of them, however, had told no-one at all. The partners of these fathers intended to tell their child at some point in three cases (age not stated) or were undecided.

Factors that influenced these men's decisions to never tell (Table 6.3) were varied and included:

i) parental factors such as parents unable to agree on decision
ii) concern about their child's reactions/feelings
iii) wider world concern, for instance that their child would reveal the method of conception to others or concern about child's acceptance within the family's culture (moral, ethical or religious background)
iv) other reasons, such as there just being no need for their child to be told, unless there were health implications.

c) Reasons given by parents who are undecided whether to inform their child

Factors given by the 21% of fathers and 16% of mothers who were undecided included: parental factors, child reactions/feelings and wider world Other factors included the wish to wait until sex education as a whole was discussed and concern that the child was too young for the parents to have given serious thought about approaching the topic (Table 6.4). Seven parents also commented that they felt the subject was not important or relevant to the child.

Parents who intended to tell their child in the future were asked if it would be helpful to have child-friendly literature to help explain the conception to the child. A large majority (92% of mothers, 82% fathers) reported that they would find literature helpful.

Nine out of 31 (29%) undecided fathers and 10 out of 28 (36%) undecided mothers stated that they would tell their child if they had access to appropriate child friendly literature that explained the topic.

Table 6.3 Reasons given for not wishing to inform child of mode of conception

Father	Parental factors	Child reaction/feeling	Wider world	Other[abbreviated]
1				Yes[a]
2			Yes	
3	Yes	Yes		Yes[b]
4				Yes[c]
5		Yes	Yes	
6		Yes		

[a] I see no reason for them to know unless it has health implications

[b] Telling the child adds to life "baggage". I would not deny it, but see no merit in telling the child.

[c] No reason to know. If we feel she needs to be told in the future then we may decide differently.

Table 6.4 Reasons given for indecision about whether or not to inform child of mode of conception

Reasons for indecision	*Mothers*		*Fathers*	
	n=28	(%)	*n=31*	(%)
Parental factors	16	57%	13	42%
Child reaction/feelings	15	54%	13	42%
Wider world	7	25%	6	19%
Child too young	21	75%	22	71%
Not discussed sex education	15	54%	10	32%
Not relevant / important	3	11%	4	14%

6.3.7 Non-responders

The response rate was as expected for this type of postal survey (Cummings *et al.* 2001; Boreham *et al.* 2003) using a single mail out. No socio-demographic differences between non-responders and responders could be found.

We found that few parents who responded said that they never intended to tell their child. It is possible that parents holding this view are less likely to respond.

6.4 Discussion

This is the largest study of parental attitudes towards informing their IVF or ICSI conceived child of their mode of conception. The majority of parents who responded had already disclosed, or wished in the future to disclose, details about the conception method to their child. The same proportion of mothers and fathers planned to inform their child in the future, but fathers on average would tell their children slightly later, at about 10 years rather than 8-9 years for mothers. It is of note that, in a much smaller study of 8-9 year olds, the percentage of parents who were undecided, who intended to tell at some point and who had already told are very similar to this study. That study also found that parents who had informed their child had done so between 4-8 years (Colpin and Soenen 2002).

Children who had already been informed, by the age of 5, about their conception status were more likely to have been told by their mothers. However, the questionnaire did not ask how the information was given or in what detail. The very young age cited by some respondents suggests that the question was interpreted as the age at which the subject was first introduced and may not represent an age at which the child fully understood the information. Further research with children could explore this.

Parental decisions about whether or not to inform their child may be influenced by their decision to reveal the method of conception to others. Parents who did not mind who knew the child's conception status (56% mothers and 53% fathers) were more likely to have already or intended to inform their child. The remaining parents may therefore have been more selective in their choice of confidants. This is illustrated by parental reports that issues outside the family (wider world) contributed to their decision. These parents expressed concern that their child would reveal the method

of conception to others or concern about their child's acceptance within the family's culture (moral, ethical or religious background). It has been reported that some cultures outside the UK are more secretive about disclosing conception information and more uncertain about whether to inform their child (Cook *et al.* 1997).

However, the majority of parents in our study who were concerned about wider world factors had told somebody and had not kept the child's conception method completely secret. Secrecy has been shown to be detrimental to family relationships, creating boundaries between those who do and do not know. Holders of family secrets may experience anxiety about the possibility of disclosure and find discussion of related topics uncomfortable. If the secret is subsequently disclosed, the previously unaware party may feel that their trust has been violated (Karpel 1980).

Almost half the parents (54% mothers and 42% fathers) also stated that they were concerned about their child's response or concern that child would feel different to siblings or peers. In addition, the presence of siblings was associated with less disclosure. Possibly parents are reluctant to highlight differences between children. However, general parental fear that their child may be singled out if their mode of conception was known by others has been reported previously (McWhinnie 1996) and this concern was also reported by three of the six fathers in this study who did not wish ever to inform their child.

Undecided parents were most concerned that their child was too young. All the children of families in this survey were under the age of 6.5years. The parents were not asked if the current age of the child was relevant to their future decision about revealing mode of conception. It may be that parents wish to wait until their child has developed further and asks questions for him/herself. The majority of parents who stated that their child's age was a factor also had other reasons for non-disclosure.

Some parents related the decision to tell their child with the timing of sex education. The discussion of sex education between parents and children has been shown to be difficult in cases of natural conception (Colpin and Soenen 2002) and it may be that these difficulties are compounded by the need to explain assisted conception. The optimal age to discuss sex education is not established, but charities such as the

family planning association (FPA) advocate approaching the subject from an early age (Family Planning Association 2003). Many parents may rely on the educational system to teach their child this topic and in the UK the discussion of assisted reproduction is suggested in the national school curriculum for 11 year olds (Qualification and Curriculum Authority 2003). This may teach children the basic facts, but the child will not discover how they were conceived from this source.

Many parents are unsure how to approach the subject of assisted reproduction with their child. Parents may not know where to turn for this advice. A previous study found that 24% of parents felt that their fertility clinic could have been more helpful regarding the issue of disclosure (Greenfeld *et al.* 1996). This study found that 29% of undecided fathers and 36% of undecided mothers said they would tell their child if they had access to appropriate child friendly literature that explained the topic. In addition, almost all those who intended to tell their child (82% of fathers and 92% mothers) said they would welcome appropriate child friendly literature.

The evidence gathered in adoption studies (Hoopes 1990) is that informing children early in life of their origins contributes to the formation of successful identity and subsequent wellbeing. The simple intervention of provision of child friendly literature may therefore be helpful to many parents and beneficial in the long-term for their children. It is likely that informing children of their assisted conception at a young age may also be advantageous. History has a tendency to repeat itself and it may be that, as with adoption studies, surveys of the attitudes of IVF conceived adults in the future may be the most accurate method of establishing how and when the IVF conceived child should be told, and what they would benefit from knowing.

6.4.1 Child friendly literature source for families

The results of this study have revealed that parents of IVF children do not have access to resources that would aide them when discussing IVF conception with their children. The provision of child friendly literature may therefore be helpful to many parents.

On the basis of this survey, the researchers (Catherine Peters, Xenya Chrysostomou, Alastair Sutcliffe) have produced a booklet to aide parents in their discussions with

their child. The majority of parents who stated an age at which they intended to tell their child, indicated that they would do so at an age less than seven years old (figure 6.1). The booklet is therefore aimed at a children aged 7-8 years. It includes a description of natural conception, IVF and ICSI, with equal weight of importance to each mode of conception.

The booklet has been reviewed by directors of fertility clinics and parents of IVF children and has been published by the medical illustration department at the Royal Free Campus of UCL (appended).

Chapter 6

KEY POINTS

- **The majority of parents wish to disclose conception method to their child**
- **The average age for planned disclosure was 8-9 years for mothers and 10 years for fathers**
- **Very few parents had told no-one about their child's conception method**
- **Parents who did not mind who knew their child's conception method were most likely to inform their child**
- **Undecided parents were most concerned that their child was too young**
- **54% mothers and 42% fathers were concerned about their child's response to the disclosure**
- **Parents of ICSI children were more likely to inform their child than parents of conventional IVF children**
- **The presence of siblings was associated with less disclosure**
- **Parents are unsure how to approach the subject of assisted conception with their child**
- **Many parents would tell their child if they had access to child-friendly literature**

CHAPTER 7: GENOMIC IMPRINTING SURVEY

7.1 Background of survey

The proposed mechanism of genomic imprinting and the possibility that this process may be interrupted during assisted conception has been discussed in chapter 1 (1.9). The potential risk of an increased occurrence of imprinting disorders after IVF is of concern and performing population studies to evaluate this risk is difficult.

The Human Fertilisation and Embryology Authority (HFEA) hold confidential information regarding the identities of IVF children, but the purpose of this registry is not for research. IVF clinics have some data regarding the families that they have treated, but may be reluctant to participate in studies which may show any potential detrimental effects from their treatments.

If IVF families cannot be effectively traced and contacted for this type of study, an alternative approach is to contact families of children with imprinting disorders (for example Beckwith-Wiedemann syndrome) and establish how many parents had assistance with conception. The most effective way to contact these families is through the family support groups.

7.2 Study type

This study was a survey of children with Beckwith-Wiedemann syndrome (BWS). The aim of the survey was to establish the number of BWS children who were conceived after assisted reproductive therapy (ART) to get evidence of an increased risk of BWS after ART.

7.3 Ethical approval

MREC approval was obtained with permission to contact families through family support groups and genetic clinics.

7.4 Methods

7.4.1 Contacting families through the Beckwith-Wiedemann support group

The Beckwith-Wiedemann support group was approached and agreed to participate in our study. A study pack was produced which contained the following:

- A parent information letter containing a full explanation of the study aims and methods
- A questionnaire designed by the candidate and Dr Alastair Sutcliffe, asking parents to provide information about their child's method of conception ie natural, conventional IVF, ICSI, frozen embryo, donor egg or sperm and other. The questionnaire also inquired about any history of infertility and any family history of illness / medical conditions (see appendix 7).
- A consent form ensuring that the aim of the study is understood and giving permission for us to contact the clinician involved with the affected individual if necessary.
- A reply-paid envelope to encourage a good response.

The support group organiser, Mr Bob Baker, sent a study pack to all the families on the support group mailing list who had a family member with Beckwith-Wiedemann who was under 21 years of age. This age cut off was chosen because there were very few ART conceived children born prior to this age group.

Returned questionnaires were assessed by the candidate and the responses were recorded. Mr Baker was not given any information about the questionnaire responses other than the names of families who had replied. He was then able to send reminders to those families who did not reply after the first mail out. The response rate was recorded.

From these responses, it was possible to estimate the incidence of children with BWS that were conceived after assisted conception.

7.4.2 Collaboration with other researchers

Researchers at the Medical and Molecular Genetics Department, University of Birmingham, led by Professor Eamonn Maher, have been working on the identification of the genes implicated in the development of Beckwith-Wiedemann syndrome. As a result, they have a database of children with this condition. We were therefore able to arrange collaboration between our research groups.

Using this database, families who had not already been contacted through the parent support group were sent the same questionnaire. Families who conceived their child with assisted conception were noted and the response rate was recorded.

7.4.3 Identification of Beckwith-Wiedemann children who were conceived after assisted conception.

These two methods of contacting families allowed us to ascertain details of children who had been diagnosed with Beckwith-Wiedemann and who had been conceived after assisted conception.

7.5 Results

7.5.1 Assisted conception and BWS children recruited from the support group

Questionnaires were sent to 160 families belonging to the support group. We had 53 replies giving a response rate of 33%. Of these 53 responses, there were 5 children (9.4%) whose parents had assisted conception (table 7.1). Three of these children were conceived after ICSI.

Allowing for a potentially biased response rate, if the 5 ART conceived children are considered in the context of the 160 families to whom questionnaires were sent, the percentage of assisted conception children is a *minimum* 3%.

The age range for all responses was 8 months – 21 years. All the children of respondents who had been conceived after assisted reproductive therapy were less than 8 years old ie conceived between 1995 and 2002.

7.5.2 Assisted conception and BWS children recruited from Birmingham medical genetics centre

The researchers in Birmingham have a database of 141 BWS patients. Of these, there were 78 cases with imprinting defects (as opposed to uniparental disomy and other mechanisms) of which the conception status of 34 individuals was known (including those children who responded to the support group survey).
Questionnaires were sent to the remaining 44 cases and there were 19 replies (response rate 43%). 3 cases were conceived after ART; 1 ICSI; 1 IVF; 1 LHRH injection given to induce ovulation.

The age range for the responders to the Birmingham BWS survey was 1 year to 39 years. All those with BWS who were ART conceived were born after 1989.

7.6 Analysis

Between the two surveys, a total of 8 cases of children with BWS were found to have been conceived after ART. 5 of these children were conceived after IVF/ICSI, the oldest of which was born in 1998 and the youngest in 2002. 3 children were conceived after ovarian stimulation, the oldest child was born in 1989 (LHRH injection given to mother) and the youngest in 1998.

The records kept by the HFEA have been collated by differing methodologies and it is difficult to calculate the number of ART livebirths per year (table 7.3). However, the HFEA data states that during 1991-1999 there were 44,211 livebirths after ART (+1106 pregnancies with unknown outcome). Between 1999 and March 2002, there were 15,929 live births (+ 6107 unknown outcomes). This gives a total of livebirths between 1991 and 2002 of 60,140 (with 7213 unknown outcomes). In addition there were 1213 cycles of IVF for which there is no data (lost to follow up). In these cases, there may or may not have been a pregnancy and the outcome of any potential pregnancy is unknown. There is no data for outcomes after superovulation only.

The total number of livebirths in England and Wales for each of the years between 1991 -2002 is shown in table 7.4, as published by the Office of National Statistics and the Information Service Department of Scotland.

The number of children conceived after IVF/ICSI techniques during these years was 68,566 (data from HFEA including unknown outcomes). Over the same time period 8,395,627 children were born in the UK (UK office national statistics & information services department, Scotland). This means that 0.8% of births during this period were IVF/ICSI conceptions.

In theory the number of children conceived after ICSI and IVF in our support group cohort of children with BWS should be 0.8% of 160 or 1.3. We had 5 children (3%) conceived after ART. If we exclude those conceived after superovulation (the HFEA figures do not include these children), we have a *minimum* of 3 children (2%).

Our findings of an increase in children conceived after ART in the BWS population is corroborated by the data from the Birmingham centre. This centre now has a database of 141 individuals with BWS, of whom 78 are known to have imprinting defects. They now have evidence of at least 9 cases of BWS with ICSI/IVF and 2 further cases of superovulation. For a population of 141 people with BWS of all genetic background, this gives a 7.8% incidence of ART in the BWS population. Given that many of the individuals on the database were born before IVF was widely used, the figure may be even higher. The percentage of ART in the groups of BWS children with imprinting defects is 9/78 (11.5%).

Table 7.1 Data from BWS support group survey

Sex of child	Child DOB	Maternal DOB	Paternal DOB	Type of conception	Family history
Male	18/03/2002	04/06/1968	10/09/1967	ICSI	No
Male	31/05/2000	03/11/1963	22/03/1962	ICSI	No
Female	20/04/1998	18/12/1968	29/06/1967	Superovulation	No
Male	30/05/2001	10/02/1962	15/11/1958	ICSI	No
Male	05/06/1995	16/10/1956	11/11/1950	Clomid	No

Table 7.2 Data from Genetic centre BWS survey

Sex of child	DOB of child	Maternal DOB	Paternal DOB	Type of conception	Family history
Male	24/03/2002	25/05/1970	29/07/1968	ICSI	No
Female	31/05/2002	16/04/1969	01/10/1965	IVF	No
Female	05/12/1989	24/02/1959	25/05/1956	LHRH injection	No

Table 7.3 HFEA data for IVF births 1991-2002

Database*	Treatment	Births	Lost to follow up[a]	Missing Outcome[b]
Sybase	IVF	14,017	82	5,355
Sybase	DI	1,912	17	752
FoxPro	IVF	33,406	836	732
FoxPro	DI	10,805	278	374
Total		60,140	1,213	7,213
Maximum possible births			68566	

[a] HFEA are unclear whether there was a pregnancy or subsequent birth

[b] HFEA are unaware of outcome of known pregnancy

*Sybase database includes 1999 to March 2002, FoxPro database includes 1991 to 1999, so effectively this is numbers of treatment cycles from HFEA's start in 1991 to March 2002.

Table 7.4 Livebirths in the UK between 1991-2002

Year	**Livebirths England and Wales**	**Livebirths in Scotland**
1991	699217	66740
1992	**689656**	**65046**
1993	673467	63293
1994	**664726**	**61656**
1995	648138	60051
1996	**649485**	**59308**
1997	643095	59440
1998	**635901**	**57319**
1999	621872	55147
2000	**604441**	**53076**
2001	594634	52527
2002	**596122**	**51270**
Total	7,690,754	704873
UK total		**8,395,627**

7.7 Discussion

This survey, although small, is further evidence that there is an association between assisted conception and Beckwith-Wiedemann syndrome (BWS). Determining the exact incidence of children conceived after assisted reproduction who have BWS is difficult. BWS has a range of clinical manifestations, many of which are subtle and the diagnosis is often missed. In addition, there is no statutory body that collects prospective data on the outcome of IVF children.

Contacting IVF families retrospectively to ascertain the incidence of BWS in this population would require a very large number of families given that the incidence in the general population is believed to be 1 in 13,000. This method is therefore currently impossible in any country. The use of the BWS support group to investigate the link between this rare condition and ART was an alternative method. The support group has 160 members, which is a relatively large sample of the BWS population. It is not possible to assess if there are any differences in the families of children with BWS who do or do not join this society.

For our analysis, the number of children conceived after IVF techniques was based on HFEA data and included pregnancies with unknown outcome. We have assumed that the outcome for each pregnancy was a live birth and this is likely to overestimate the number of ART children born in the UK during this time period. It is therefore possible that the percentage of children conceived after ART in the general population over this period is lower than the 0.8% calculated.

Allowing for methodological difficulties, there is clear evidence of an increase in BWS in children conceived after ART. This finding is supported by the few other studies published so far (DeBaun *et al.* 2003; Maher *et al.* 2003; Gicquel *et al.* 2003). The next step is to investigate whether the genetic mechanism for BWS in ART children differs from that of BWS in the general population.

All the ART children recorded to have BWS in our survey are under 8 years old. This may be an effect of the increase in use of ART in the last 10 years. However, it is also of note that the majority of ART children in our survey were conceived after ICSI which was introduced in 1992. It is unclear if there was any other change in

ART around this time, perhaps differing drugs used for ovarian stimulation or changes to the culture media.

The response rates of the two surveys were low (33% support group, 43% genetics centre). The genetic centre response rate may be better because the families involved had already consented to research in the past. However, all the potential response rate bias in the two surveys is likely to underestimate the number of BWS who were conceived after ART. The phenomenon may be much greater.

7.8 Further research

There have also been reports of an increase of Angelmans syndrome in ART children (Cox *et al.* 2002). To assess the risks of this and other genomically imprinted syndromes, a UK and Ireland collaborative group has been established. This group was named Assisted Reproduction Therapies and Imprinting Disorders (ARTID) and includes:

Professor Eamonn R Maher Professor of Medical Genetics (expert on Beckwith-Wiedemann syndrome), University of Birmingham

Dr Karen Temple, Consultant Geneticist, (expert on Transient Neonatal Diabetes), Wessex Clinical Genetics Service, Southampton

Dr Jill Clayton-Smith, Consultant Geneticist (expert on Angelman Syndrome) Department of Clinical Genetics, Manchester

Dr Wolf Reik, (expert in the organisation and expression of imprinted genes clusters, and their role in fetal development, growth, and disease), Cambridge

Dr Louise A Brueton, Consultant Geneticist, University of Birmingham

Dr Louise Wilson, Consultant Geneticist, Great Ormond Street Hospital, London

Dr Willie Reardon, Consultant in Clinical Genetics, Republic of Ireland

Dr Alastair Sutcliffe. Senior Lecturer in Child Health, University College London

Professor Chris Barratt, Department of Obstetrics and Gynaecology, University of Birmingham

Dr Sarah Bowdin, Genetics registrar (SpR), University of Birmingham

Dr Catherine Peters, Paediatric Research Registrar (SpR), University College London

This group have arranged for the same questionnaire used for the BWS survey to be sent to families of children with Angelmans syndrome, Prader-Willi syndrome and Transient Neonatal Diabetes. The results of these surveys are awaited.

CHAPTER 8 – SUMMARY OF THESIS FINDINGS

-MAIN CONCLUSIONS FROM THE STUDIES IN THIS THESIS

8.1 Child and family development study

The results of these studies are generally reassuring for parents of children conceived after ICSI or IVF. The study has addressed the hypotheses stated in chapter 2 and these are discussed below:

Hypothesis 1: children born using ICSI are expected to display the following in comparison with both IVF and naturally conceived children:

- *a greater occurrence of minor physical anomalies*

The proportion of total major and minor congenital malformations was significantly higher in the ICSI and IVF groups compared to the naturally conceived group. Specifically, there was an increase in genitourinary abnormalities in ICSI and IVF boys. The numbers involved are small and further studies would be needed to establish this.

The hypothesis was confirmed (within the limited numbers of this study) in terms of comparing ICSI children with naturally conceived children, but the IVF group was found to be have the same outcome as the ICSI group, possibly suggesting a link with the genetics/nature of the parents needing fertility treatments or a common part of the assisted reproductive process (ovarian stimulation / culture media) rather than ICSI per se.

- *no difference in overall physical health*

From this study of children at 5 years of age, no differences were found between conception groups for hospital admissions or medications for the children which could be viewed as objective evidence of similar health between groups. However, there was a higher rate of childhood illness reported by parents of ICSI and IVF children. This may represent a genuine increase in illness in these groups or an increase in parental anxiety and perception in the parents of assisted conception children.

The study found that although ICSI children were mainly born at term, they were born at a slightly earlier gestation than control group children (39^{+1}/40 ICSI vs 39^{+6}/40 control). However, neonatal outcomes were similar between groups with no differences in neonatal illness or birthweight.

- *a greater occurrence of fine and gross motor difficulties*

The neurodevelopment of 5 year old ICSI, IVF and naturally conceived children was assessed in this study using the WPPSI scores and the McCarthy motor skills test for motor development. There were no differences seen between the three groups in any areas of neurodevelopment and the null hypothesis has not been rejected.

There had been concerns raised about the neurodevelopment of ICSI children with some groups finding evidence of poorer neurodevelopment (Bowen *et al.* 1998) and others finding no differences (Bonduelle *et al.* 1998b; Sutcliffe *et al.* 2001). These studies examined children aged 12-26 months and used different development scales. The results of this study of children aged 4½ -5½ is likely to be more representative of children's abilities as they are generally more cooperative at this age and the tests used are more predictive of future abilities. This study also had the advantages of a larger cohort and the assessment of neurodevelopment was performed by a psychologist who was blinded to the mode of conception of the children involved. These results are, again, reassuring to parents of ICSI and IVF children.

- *a greater occurrence of difficult temperament and emotional or behavioural problems*

Parents of ICSI children reported some aspects of more difficult temperaments in their children compared to parents of IVF and control children. For ICSI children, their mothers reported more difficult levels of rhythmicity and intensity. Their fathers reported them to be more negative in mood and both parental groups reported decreased adaptability.

However, despite the more difficult temperament reported by the parents of ICSI children, these children were found to have lower levels of externalising behaviours such as temper tantrums and aggression compared to controls (IVF children were reported to have similar levels to ICSI children).

These outcomes may result from differences in parenting styles between groups. It has been suggested that parents of assisted conception children have more intense relationships with their child and are more over-protective (Golombok *et al.* 2001; McWhinnie 1996). The parent-child relationship is discussed below.

Hypothesis 2: families of children born using ICSI will experience the following:

- *more commitment to parenting and less commitment to work*

Mothers of ICSI children were found to have a much higher commitment to their role as a parent than mothers in the other two groups. There was a trend towards a lower commitment to work in this group of mothers, but it was not significant. However, IVF and ICSI fathers had lower commitment to work than fathers of control children.

It is perhaps unsurprising that the maternal commitment to parenting was high, given that these mothers have undergone numerous investigations and invasive treatments in their quest for conception and motherhood. After the birth of their child, these mothers may have a need to prove to themselves (and others) that they can fulfil this motherhood role well.

- *more stress in the parent-child relationship*

Mothers of ICSI children tended to have less parental stress than control mothers. In addition, although parents of ICSI children reported more difficult temperaments in their children, they were less likely to report feelings of aggression/hostility than parents of controls. This may be because these parents are more committed to their roles as parents (see above). Alternatively, the increased age of the assisted conception parents may lead to differences in tolerance or a more realistic expectation of parenthood. ICSI and IVF parents are also more likely to be married and may provide additional support for each other if the child displays evidence of difficult temperament.

Mothers of ICSI children, together with fathers of ICSI and IVF children also reported less symptoms of rejection from their child. For ICSI fathers, this finding is supported by the assessment of the family relationships from the child's point of view. ICSI children reported more positive interaction with their fathers than other children. There may be a difference in the ICSI fathers' interactions with their

children and, if so, this may reflect the fact that many ICSI children are conceived by this method because of male factor infertility.

- *more stress in the marital relationship*

There were no differences between parental groups for any of the dyadic adjustment subscales, indicating that there were no differences in parental relationships between conception groups. The null hypothesis has not been rejected.

- *more mental health problems in parents*

This hypothesis was also disproved. There were no differences between parental groups for general health, but ICSI and IVF mothers reported less symptoms of severe depression than control mothers. ICSI mothers also scored lower on the parenting distress index. There were no differences between fathers in each conception group for any of these variables.

8.2 Parental attitudes towards disclosing the method of conception of their ICSI/IVF child

Studies into disclosure of mode of conception in assisted conception have focused mainly on children conceived after donor insemination. There are fewer studies into disclosure of mode of conception in IVF or ICSI parents who are both genetically related to their children. Those studies that have investigated this have been very small and have not investigated the underlying reasons for parental decision in this area. From the study information available, we set out the hypothesis below and aimed to examine parent's motives for their decisions.

Hypothesis: Parents of children conceived after IVF are reluctant to inform their child about their mode of conception.

We found that the majority of parents intended to tell or had already told their children how they were conceived. 16% mothers and 25% of fathers were undecided or sure that they did not want to tell.

The main barriers for parents to disclosing conception methods were difficulties in deciding how and when to approach the subject with their child, and concerns about the reaction of the child and others once the disclosure had taken place. There is little

information available for parents to guide them how and when may be best to tell their child. This type of information may be hard to provide, as what may suit one family and child may be different for another family and child. However, as a result of this study, the authors have produced a child-friendly booklet aimed at helping parents to explain to their child how they were conceived. There may also be a place for clinics to provide further counselling on this topic, perhaps on a longer term basis.

8.3 Genetic imprinting and assisted conception

Hypothesis*: *Children conceived after in-vitro fertilization techniques are at increased risk of developing conditions caused by genetic imprinting defects than their naturally conceived peers (eg Beckwith-Wiedemann syndrome).

Our survey indicates that there is an association between Beckwith-Wiedemann syndrome and assisted reproductive therapies. This supports our hypothesis and the findings of other researchers (DeBaun *et al.* 2003; Gicquel *et al.* 2003; Maher *et al.* 2003).

It is of note that the majority of cases of ART conceived children with BWS were under the age of eight. This may be an effect of the increased use of IVF technology or it may represent a link with ICSI, which was developed in 1992. It could also be linked with a change in the culture media used or drugs given. Further research is needed.

8.4 Conclusions

The studies and literature reviews in this thesis are generally reassuring, in terms of physical and neurodevelopmental health of children aged 4-5 years. The studies also show evidence of generally positive relationships in families of ICSI and IVF children. In addition, the majority of families of assisted conception children wish to disclose conception method to their child but unsure how to do this and require additional support. As a result of these findings, we have produced a booklet to help families of assisted conception children who wish to tell their child about their method of conception.

However, there remain areas of concern. The studies suggest that there is an increase risk of congenital abnormalities after ICSI and IVF. The numbers involved in these studies are small and therefore large scale multi-national studies are required to assess whether there is a true increase and whether there is any pattern in the nature of the congenital abnormalities.

There is also evidence of an increased risk of BWS in children conceived after assisted reproduction. This phenomenon is interesting and the genetic mechanism is now being studied by other groups. It remains to be seen if other syndromes caused by defects of genetic imprinting are also increased after ART and the candidate is involved in ongoing research in this area.

The studies in this thesis examine the outcomes of children conceived after conventional IVF and ICSI and who are their genetic progney. However, many of the medical findings may be relevant to ART children who do not fit the criteria for entry into our studies.

True reassurance of the safety of these technologies will only be achieved by following these children throughout their lifetime. These study findings, and the areas of concern highlighted above, should be taken into consideration by those undertaking future research involving ART children. This is discussed in the following chapter.

CHAPTER 9 – FUTURE RESEARCH RELEVANT TO THIS THESIS

9.1 Areas for future research

9.1.1 Long term follow up of ICSI children

The developmental / IQ outcomes of children conceived after ICSI as measured in this study are likely to be representative of future attainment and do not indicate any cause for future concern in this area. In addition, the physical health of the children was generally reassuring, although the frequency and nature of congenital abnormalities may be of concern and warrants much larger studies to ascertain the true risks of congenital abnormalities associated with ICSI and IVF.

It is also possible that other medical problems, such as cancers, may be associated with ICSI and may arise at a later age, possibly even in adulthood. This can only be assessed by future studies, either by following up an ICSI cohort such as this one or by entering mode of conception into medical registries such as the cancer registry. This latter method would allow a prospective study of children (and adults of the future) with cancer and assessment of the relationship of cancers to mode of conception (see 8.3.5).

9.1.2 Effects on fertility of 2nd generation

The first IVF conceived child, Louise Brown is now in her twenties. It is likely that IVF children of her generation are planning to conceive children of their own. The fertility of this generation has yet to be established. The oldest ICSI children are just entering their second decade and therefore assessing the fertility of this group of individuals remains several years in the future. There is some evidence that a small proportion of ICSI conceived boys whose fathers have a Y chromosome deletion may inherit this deletion and the associated infertility (Kent-First *et al.* 1996). We do not know whether other sub groups will be affected.

9.1.3 Assisted Reproductive Therapy and Imprinting Disorders (ARTID) group

This group was set up to research the incidence of Beckwith-Wiedemann, Angelmans syndrome, Prader-Willi syndrome, Silver-Russell and Transient Neonatal Diabetes in children conceived after assisted reproductive therapies. The preliminary findings for Beckwith-Wiedemann syndrome have been reported in this thesis and

there is evidence to suggest that this condition is increased in the population of children conceived after assisted reproduction. The group will continue to monitor the incidence of these and potentially other imprinting disorders.

9.2 The future of assisted conception and ethical considerations

9.2.1 Embryo replacement policies

Embryo replacement policies have been discussed in chapter 1, but the debate is ongoing. Several studies have shown that the high rate of multiple pregnancies in IVF conception is responsible for the increase in prematurity, low birth weight and perinatal mortality (Friedler *et al.* 1992; Hershlag *et al.* 1990). These high rates of multiple pregnancies are mainly the result of the number of embryos transferred. Limiting the numbers of embryos transferred would limit the multiple pregnancies rate and the associated morbidity.

The UK Royal College of Obstetricians and the Human Fertilisation and Embryology Authority (HFEA) have recommended two-embryo replacements in all women less than 40 years. Several authors advocate the replacement of only one embryo at a time (Gerris and Van Royen 2000; Hazekamp *et al.* 2000; Templeton 2000). Perhaps the pressure to replace more than one embryo will be reduced as IVF techniques improve. This is illustrated in the United States where there have been consistent decreases in both the number of embryos transferred per cycle and the percentage of pregnancies with three or more fetuses, as well as a consistent increase in the percentage of live births per cycle (Jain *et al.* 2004).

For the present, and in the future, the outcome of a healthy IVF conceived child (rather than just the attainment of a pregnancy) must be the most important criteria when assessing the success rates of IVF.

9.2.2 NHS provision of treatment

There have been inconsistencies in the provision of NHS fertility treatment throughout the country. The UK Secretary for Health announced in February 2004 that all areas of the UK must provide at least one cycle of IVF free on the NHS by April 2005, with a longer term aim to provide at least 3 free cycles (DoH 2004). The

net effect of the increased provision of free fertility treatment in some areas may be an increase in IVF conceived children.

9.2.3 Introduction of new techniques

If new techniques are introduced, then lessons should be learnt from the study of ICSI children so far. Firstly, it is necessary to establish the safety of existing methods. Secondly, follow up studies should be introduced in a prospective manner as soon as the new technique is commenced.

An example of a technique which would benefit from a prospective study is the recently introduced Preimplantation Genetic Diagnosis (PGD). This method of diagnosing genetic disease involves the removal of 2 out of 8 cells for genetic testing. The remaining cells are thought to be pluripotential and will continue to divide until a critical cell mass is reached. However, as yet no studies are in place to monitor the children who are born after this form of genetic testing.

9.2.4 Cloning

(a) Reproductive cloning

The first attempts at cloning were developed in amphibians in the 1950's (Briggs and King 1952). This involved the transfer of an embryonic nucleus into an unfertilized oocyte which had had its nucleus removed (an ooplast). The cloning of the sheep, Dolly was the first successful adult somatic cell nuclear transfer (SMNT) (Wilmut *et al.* 1997). Since Dolly, several other mammals have been successfully cloned including cows, mice, goats, pigs, rabbits and cats.

A report in Science previously reported the cloning of a rhesus monkey, but the technique used involved splitting an embryo rather than transfer of genetic material from one cell to another. The 8 cell blastocyst was split into 4 pluripotential two cell groups. These "two-cell embryos" were transferred to a surrogate mother. Although there was a quadruplet pregnancy, there was only one live birth (Chan *et al.* 2000).

However, no primate has been cloned using adult SMNT, making the reproductive cloning of humans appear some way off. Cloned primate embryos lack proteins that enable the chromosomes to line up on the cell spindle before separation of the

chromatids during mitosis. The chaotic alignment causes aneuploidy in the resulting cells (Simerly *et al.* 2003).

In those mammals where SMNT cloning has been achieved, the technique remains inefficient and has many associated difficulties to overcome:

- Poor pregnancy success rates

  Pregnancy rates are lower than expected and there is a high rate of miscarriage throughout the first and second trimesters (Hill *et al.* 1999; Kato *et al.* 2000). Kato found that only 14% of transferred bovine SMNT derived blastocysts developed into calves. There is a higher rate of fetal and placental abnormalities and this is thought to contribute to the increase in pregnancy loss and the reduced viability at birth (Hill *et al.* 1999; Wilmut *et al.* 1997).

- Congenital abnormalities

  After birth there is a high rate of respiratory and cardiovascular abnormalities leading to an increase in neonatal death (Hill *et al.* 1999) and those surviving clones may suffer from renal, neurological and immune deficiencies (Wilmut *et al.* 1997). Some cloned cattle have been found to be oversized "large offspring syndrome" although this phenomena is seen after the use of other reproductive techniques (Young *et al.* 1998).

- Premature ageing and telomere length

  There is some concern that cloning techniques can lead to premature ageing. This may occur through telomere shortening. The telomere is a terminal chromosomal cap sequence that protects chromosome DNA termini from degradation, fusion and recombination during mitosis (Mollard *et al.* 2002). These telomeres shorten with each replication until a critical length is reached which is associated with subsequent cell death. Dolly the sheep was found to have reduced telomere lengths that corresponded to the lengths of those of her donor (Shiels *et al.* 1999). There was evidence that Dolly also developed early onset arthritis that may have been associated with premature ageing (Williams 2002). Subsequent experiments have indicated that in some cases the telomere lengths can be reset although the mechanism is not understood (Mollard *et al.* 2002).

- Genomic Imprinting abnormalities

  The mechanism of genomic imprinting and a study of the effects of this process in IVF children are discussed in chapters 1 and 7. In natural conception, after fertilisation of an egg by the sperm, both genomes undergo general demethylation until the pre-gastrula stage when cell-specific methylation patterns are established. There is evidence that there is incomplete erasure of epigenetic markers during SMNT and this may be responsible for the high incidence of congenital abnormalities and miscarriages in cloned animals (Mollard *et al.* 2002).

Despite this, there are several groups participating in research aimed at cloning a human. In November 2002, a company called Clonaid claimed to have cloned the first human baby, a girl nicknamed "Eve". These unsubstantiated claims have generated an increased public debate on the issue.

(b) Therapeutic cloning

Therapeutic cloning uses the same SMNT methods and enables isolation of embryonic stem cells prior to the development of the blastocyst into a fetus. These embryonic stem cells are hoped to provide a mechanism for developing replacement cells, tissues and organs for individuals with disorders such as diabetes, heart disease and organ failure.

Adult stem cells found in bone marrow are also being investigated for their pluripotential properties as a possible alternative to the use of embryonic cloning which many find unethical. Other researchers are investigating the use of Olfactory Ensheathing Cells (OEC) which ensheath the axons of olfactory receptor neurons. These cells have the ability to support neurogenesis throughout life and there is evidence that when they are transplanted to the damaged spinal cords of rats, they promote spinal cord repair. Studies are currently investigating the use of OEC's to promote repair of spinal cord damage in humans (Barnett and Riddell 2004). However, these alternative research methods lag behind the embryonic stem cell research.

(c) Legal aspects to cloning

In the UK, therapeutic cloning is legal, but reproductive cloning is not. Many countries do not have any laws in this in this area. A move to ban human cloning was rejected in the European Parliament in November 2002, who instead deferred the debate. It is therefore up to individual countries to decide.

(d) Medical ethics of cloning

There are many ethical arguments based on moral and religious beliefs. However, medical ethical arguments are based on the ethics of running clinical trials. Hill et al summarised the issue in the following quote:

> "The prospect of immediately producing cloned human embryos for transfer is akin to deliberately administering drugs during pregnancy that are known to cause a high rate of abortions and congenital abnormalities. It is simply not ethical to attempt to produce and transfer cloned human embryos when the same technique is known in animals to have a high risk of causing an abnormal fetus.....The traditional first step in evaluating a human medical procedure or drug is to first prove its safety in animals, then when safety and efficacy studies are positive, to proceed to initial clinical trials".
>
> (Hill *et al.* 1999)

9.2.5 Assisted Reproduction methodology and regulation

Currently the HFEA is a statutory body which regulates, licenses and collects data on fertility treatments such as IVF and donor insemination, as well as human embryo research, in the UK. However, the many individual fertility clinics are private businesses which are run in competition with each other. There are no universal protocols and the methodology used can differ between clinics. For instance, clinics may use different culture media in which to place gametes and embryos.

The clinics record information on outcomes and techniques used but not necessarily information on culture media and length of incubation etc. It is possible that factors such as culture media may affect genetic imprinting and it is likely that in the future the HFEA may have to record and monitor in a much more detailed and regulated manner.

The approach of each clinic to treating a couple with IVF or ICSI is in many ways dependent on the individual embryologist. In the past ICSI was used primarily to treat male factor subfertility or failed IVF. However, it appears to be increasingly preferred by some embryologists as the first line treatment for other causes of subfertility. There is no published evidence that, in these additional cases, ICSI has a higher success rate. In addition, it tends to be more costly. This study found ICSI children to be generally healthy at five years, but in view of the fact that the first ICSI child is only 14 years old this year, compared with 26 years for the first IVF child, the longer term outcomes are unknown and it could be argued that more routine use of ICSI over other methods of ART is not yet justifiable.

9.2.6 Research methodology

The studies in this thesis, whilst producing valuable data on the outcome of ICSI children, have highlighted the difficulties of research in this area. The topic is sensitive and recruiting children and families can be difficult. The only central registry with information about all IVF children born in the UK is held by the HFEA which itself does not undertake any research. In view of the fact that IVF outcome studies are limited due to the difficulties in recruiting children, it may be necessary for the HFEA to change its remit to include research and follow up of children.

Where particular areas of concerns remain, such as the possibility of an increase in genetically imprinted conditions or cancers, other methods of follow up may need to be considered. An example would be the recording of the mode of conception of children diagnosed with cancer by the national cancer registry. Currently the organisation responsible for the UK cancer registry is unwilling to consider this because of concerns that asking parents to disclose mode of conception of children with cancer may, by implication, cause the parents to experience guilt that they are directly responsible for their child's illness.

An alternative method of following up children with rare imprinted conditions would be to use a surveillance group, such as the British Paediatric Surveillance Unit (BPSU). This unit runs a system of surveillance for specified conditions over a time period of one or two years. Consultants caring for paediatric patients report any cases of the specified conditions that they have seen each month. If an imprinted condition,

such as Beckwith-Wiedemann, was included in this surveillance, consultants reporting a case could be asked to enquire about the child's mode of conception.

This method would allow the incidence of the syndrome in the general population of children to be estimated and the proportion of children with the condition conceived by assisted conception techniques could be calculated. It would be possible to see if this proportion differed from the proportion of assisted conception children in the same age groups. The same arguments about parental guilt apply as in the case of cancer registries.

9.3 Conclusion

Since the birth of Louise Brown in 1979, IVF and other assisted reproduction technologies have helped tens of thousands of couples to become parents. The studies of these children over the years have been ad hoc and findings have varied. Discrepancies in study findings are generally due to methodological difficulties in recruiting children and families when undertaking this type of research. Despite this, many studies, including the main study in this thesis are reassuring at this point in the early lives of the ART child.

However, there are concerns raised in this thesis about the safety of ART techniques that have not yet been adequately addressed. Such areas of concern include the possibility of an increase in imprinting disorders, including some cancers; the possible increase in congenital abnormalities; and the fertility outcomes of the first generation of ART children

These areas are difficult to study well. Many syndromes caused by imprinting disorders have a spectrum of severity and thus may go undiagnosed in the child; some cancers caused by imprinting disorders may not manifest themselves until a later stage in the child's life; and the potential impact on the fertility of an ART child can only be studied when they reach adulthood and a significant proportion of them attempt to conceive themselves.

With the introduction of new technologies, scientific and ethical boundaries are pushed forward and there is a tendency to implement new techniques before fully

assessing the impact of those that have come before. The findings and discussions in this thesis lead to the conclusion that it necessary to ensure long-term follow up studies are in place and, in the UK, to ensure that the records kept by the HFEA are optimized. The use of ART in the UK and wider world is likely to increase further with an increase in the numbers of those who can access the treatments.

Those involved in providing and regulating the treatments have an obligation to the ART children and their families to ensure that any risks associated with ART are elucidated and families informed. Many couples seeking to conceive after ART are desperate for a child and may not be deterred by the knowledge of a small increased risk in certain conditions, but they should have the opportunity to be given this information. More importantly, their children have a right to know of any potential risks that their future may hold.

ABBREVIATIONS

ART	Assisted Reproductive Therapies
BMI	Body Mass Index
BWS	Beckwith-Wiedemann Syndrome
CI	Confidence Interval
DI	Donor Insemination
FET	Frozen Embryo Transfer
GIFT	Gamete Intrafallopian Transfer
HFEA	Human Fertilisation and Embryology Authority
ICSI	Intracytoplasmic Sperm Injection
IUGR	Intrauterine Growth Retardation
IVF	In-vitro fertilization
LBW	Low Birth Weight
OR	Odds ration
PGD	Pre-implantation Genetic Diagnosis
PZD	Partial Zona Dissection
SD	Standard deviation
SGA	Small for Gestational Age
SUZI/SZI	Subzonal Injection sperm
VLBW	Very Low Birth Weight
WPPSI	Weschler Preschool and Primary Scale of Intelligence

APPENDIX 1- Recruitment letter to schools

Royal Free and University College Medical School
UNIVERSITY COLLEGE LONDON
Department of Community Child Health
Royal Free Campus
Rowland Hill Street
London NW3 2PF
Tel/Fax

Head of Department: Professor Brent Taylor *PhD, MB, ChB, FRCP, FRACP*
Lecturer in Child Health: Alastair Sutcliffe *MD, MRCP, MRCPCH*
Research Psychologist:: Xenya Chrysostomou *BSc Hons.*
Research Fellow: Catherine Peters *MBChB, MRCP,MRCPCH*

Child and Family Development Study

Dear Parent,

I am a paediatrician who has an interest in children who are conceived with special help. To make a just comparison with children who are conceived naturally, I will need to meet children, like your child, who have not been conceived with fertility treatment. I wonder if you could help me in my endeavours?

If you decided to help with this research study, it would involve bringing your child to see two of my research team members, a paediatrician and a psychologist, who would assess your child. The assessment would consist of the paediatrician asking you and your partner questions with regard to your child and pregnancy. The psychologist would assess your child's development via games and puzzles. We have a child friendly room at the Royal Free where we are doing these assessments. We would also check your child's hearing, vision and measure them as part of a mere general physical check. There will be no blood tests! These assessments would be absolutely confidential and feedback about your child would be provided to you as required but no one else.

I am seeing children who are approximately five years of age. If your child is about five years of age and you are willing to help me with this study we would be delighted to see your family. I am able to cover any expenses involved in your travelling to see my team. I am also available for any further questions. I provide a free telephone advice line for families who have helped in studies to date. Parents who have helped me with similar studies in the past have found the experience useful. This would be a good chance to meet with a Paediatrician and discuss any other concerns you may have about your child.

Kindly leave with us your telephone number (s), child's name and their date of birth, as we would like to contact you if your child is the appropriate age for the study. If you have any queries, please do not hesitate to get in touch with me via any of the contact details above. Thank you.

Yours sincerely,

Dr. Alastair Sutcliffe (Lecturer in Paediatrics)

If you are interested in participating in this study could you kindly return the slip below to me at:

Royal Free and University College Medical School
University College London
Department of Community Child Health
Royal Free Campus
Rowland Hill Street
London NW3 2PF

Name and address of child: _____

_____

_____

Postcode _____

Child's date of birth: __ __ / __ __ / __ __ __ __

Gender*: Male/Female (*delete as appropriate)

Contact Number(s): _____ (home)

_____ (work)

_____ (mobile)

Nationality: _____

Ethnic Origin: _____

Could you tell us the age(s) of your child's brother(s) and/or sister(s) (if any)?

APPENDIX 2 - Letter of invitation to IVF families

Royal Free and University College Medical School
UNIVERSITY COLLEGE LONDON
Department of Community Child Health
Royal Free Campus
Rowland Hill Street
London NW3 2PF
Tel/Fax

Head of Department: Professor Brent Taylor *PhD, MB, ChB, FRCP, FRACP*
Lecturer in Child Health: Alastair Sutcliffe *MD, MRCP, MRCPCH*
Research Psychologist:: Xenya Chrysostomou *BSc Hons.*
Research Fellow: Catherine Peters *MBChB, MRCP,MRCPCH*

Dear Parents,

I hope that this letter finds you well. I am just writing to inform you about my child development study that *named IVF centre** have kindly agreed to contact you about.

I am a paediatrician who has spent the last eight years performing confidential research on children whose parents have had help with conceiving using IVF. My first work involved children who were conceived from frozen embryos. My present study involves assessing children who are first born and have been conceived after IVF, or a special type of IVF called ICSI and naturally conceived children. At the present time we are not studying twins or triplets.

This would involve developmental assessment using special games and puzzles, some of which are administered by my research Psychologist, Xenya Chrysostomou. We would ask you to fill in some questionnaires about yourselves and your child in addition to which the Paediatrician working for me (Dr Catherine Peters) would perform a brief physical check comprising measurements of height and weight, testing hearing, vision and co-ordination, and a brief general physical examination. There are no blood tests! You would of course be given verbal feedback regarding your child's assessment and have opportunities to ask about any aspect of your child's health. This assessment will be absolutely confidential.

We have now in fact started seeing some of the eldest children in the study for the second time. Some children were seen by me at aged 18 months and have come back for a repeat assessment. These children have enjoyed their assessments and the

families have indicated that they have found the experience useful. I now have a room allocated at the Royal Free Hospital for my study and we are able to travel to *named IVF centre** to see children.

*IVF clinician** has agreed on behalf of *named IVF centre** to confidentially contact you because your child is between age 3 and 5 years. We will contact you in writing once you have sent your personal details for us to get in touch (which we will not be aware of when you receive this letter). Some families have chosen not to tell anyone about their treatment so if we contact you by phone we will not give a reason to anyone or leave a message.

When families have IVF treatment they need to know about its safety. Without studies like the one we are conducting this question cannot be answered. So far our quality work has shown that IVF children are as healthy as their naturally conceived peers (something you may know already!). Those families who have helped in the study are given a contact advice line about their children for the future.

This study is in collaboration with four other European countries who are performing similar confidential assessments. It has been funded by the European Union and this includes a budget for families' travelling expenses. My work has been internationally recognised in providing reassuring quality reports about children conceived after some types of IVF.

I do hope you will consider helping, but if not thank you for taking the time to read this letter. If you wish to receive any further details please ring us.

Yours sincerely,

Dr Alastair G Sutcliffe
Lecturer in Child Health

If you are interested in participating in this study could you kindly return the slip below to me in the reply paid envelope.

Thank you.

Name and address of child:

_____

_____

_____ Postcode _____

Child's date of birth: __ __ / __ __ / __ __ __ __

Parent's names:

_____

_____

Contact Number(s): _____ (home)
_____ (work)
_____ (mobile)

Appropriate names entered

APPENDIX 3 - General Medical Questionnaire

Royal Free and University College Medical School
UNIVERSITY COLLEGE LONDON
Department of Community Child Health
Royal Free Campus
Rowland Hill Street
London NW3 2PF
Tel/Fax

Head of Department: Professor Brent Taylor *PhD, MB, ChB, FRCP, FRACP*
Lecturer in Child Health: Alastair Sutcliffe *MD, MRCP, MRCPCH*
Research Psychologist:: Xenya Chrysostomou *BSc Hons.*
Research Fellow: Catherine Peters *MBChB, MRCP,MRCPCH*

Patient Information **Date:** __ __ / __ __ / __ __

(a) Parents Information

Identifier: _____ Name of child: _____

Mother's details

Full name (including title): _____ DOB: __ __ /__ __ /__ __

Nationality: _____ Ethnic origin: _____

Address: _____

_____

_____

Postcode _____ Contact No: _____

Grandparents address:_____

_____

_____

Postcode _____ Contact No: _____

Marital status: Married Single Separated Living together
Divorced Widowed

Level of education: Higher degree Degree University entry or equivalent
A-level O-level/GCSE No qualifications

Occupation: _____ Social class: _____ Height: _____ cm
(Previous occupation if a full time mother)

Smoking: Yes ☐ Pipe/Cigars Cigarettes __ __ per day No ☐

Drinking Yes ☐ No. of units per week __ __ No

APPENDIX 3 - continued

Royal Free and University College Medical School
UNIVERSITY COLLEGE LONDON
Department of Community Child Health
Royal Free Campus
Rowland Hill Street
London NW3 2PF
Tel/Fax

Head of Department: Professor Brent Taylor *PhD, MB, ChB, FRCP, FRACP*
Lecturer in Child Health: Alastair Sutcliffe *MD, MRCP, MRCPCH*
Research Psychologist:: Xenya Chrysostomou *BSc Hons.*
Research Fellow: Catherine Peters *MBChB, MRCP,MRCPCH*

Patient Information **Date:** __ __ / __ __ / __ __

(a) Parents Information

Identifier: _____ Name of child: _____

Partner's details

Full name: _____

Nationality: _____

DOB: __ __ /__ __ /__ __ __ __

Address: _____

_____

_____

Postcode _____

Contact No: _____

Grandparents address: _____

_____

_____

Postcode _____ Contact No: _____

Marital status: Married Single Separated Living together
Divorced Widowed

Do you have any children from any other relationships? Yes ☐ No ☐

Level of education: Higher degree Degree University entry or equivalent
A-levels O-levels/GCSE No qualifications

Occupation: _____ Social class: _____

Smoking Yes ☐ Pipe/Cigars Cigarettes __ __ per day No ☐

Drinking Yes ☐ No. of units per week? __ __ No

APPENDIX 3 - continued

Royal Free and University College Medical School
UNIVERSITY COLLEGE LONDON
Department of Community Child Health
Royal Free Campus
Rowland Hill Street
London NW3 2PF
Tel/Fax

Head of Department: Professor Brent Taylor *PhD, MB, ChB, FRCP, FRACP*
Lecturer in Child Health: Alastair Sutcliffe *MD, MRCP, MRCPCH*
Research Psychologist:: Xenya Chrysostomou *BSc Hons.*
Research Fellow: Catherine Peters *MBChB, MRCP,MRCPCH*

Patient Information **Date:** __ __/ __ __/ __ __

(d) Pregnancy Details

Identifier: _____ Name of Child: _____

Did you take any medication whilst you were pregnant?

Yes ☐ what medication(s) _____ No ☐

Did you have any illness(es) whilst you were pregnant?

Yes ☐ what illness(es) _____ No ☐

Date of delivery: __ __/__ __/__ __ __ __

Gestation at delivery: __ __ weeks + __ __ days

Delivery: Spontaneous or induced ☐ Normal vaginal ☐
Forceps/ventouse ☐
Planned caesarian ☐
Emergency caesarian ☐
Unknown ☐

Did you intend to breastfeed your baby? Yes ☐ No ☐

Did you smoke whilst you were pregnant?

Yes ☐ what did you smoke? Cigarettes __ __ per day Pipe/Cigars No ☐

Did you drink whilst you were pregnant? Yes ☐ __ __ units per week No ☐

Did you have any adult support (financial, social etc.) whilst you were pregnant

(including your partner)? Yes ☐ No ☐

If YES, state status of adult e.g. grandmother, aunt _____

Did you work during pregnancy? Yes ☐ No ☐

APPENDIX 3 - continued

Royal Free and University College Medical School
UNIVERSITY COLLEGE LONDON
Department of Community Child Health
Royal Free Campus
Rowland Hill Street
London NW3 2PF
Tel/Fax 0171 830 2049 (**direct**) 0171 830 2440 (**dept.**) E. mail icsi@rfc.ucl.ac.uk

Head of Department: Professor Brent Taylor *PhD, MB, ChB, FRCP, FRACP*
Lecturer in Child Health: Alastair Sutcliffe *MD, MRCP, MRCPCH*
Research Psychologist:: Xenya Chrysostomou *BSc Hons.*
Research Fellow: Catherine Peters *MBChB, MRCP,MRCPCH*

Patient Information **Date:** __ __ / __ __ / __ __

(e) Neonatal Information

Identifier: _____ Name of child: _____

Sex of child: Male ☐ Female ☐

First/single child (at 2 weeks) Yes ☐ No ☐

Twin child (at 20 weeks) Yes ☐ No ☐

Did you have any adult support (financial, social etc.) after your child was born (including you and your partner)? Yes ☐ No ☐

If YES, please state status of adult e.g. mother, sister _____

Birthweight: _____ g Length at birth: _____ cm

Head circumference at birth: _____ cm APGAR: __ / __ / __

Resuscitation: Yes ☐ No ☐ Unknown ☐

Admitted to neonatal clinic: Yes ☐ __ __ days, reason _____
No ☐
Unknown ☐

Ventilated: Yes ☐ __ __ days, __ __ __ hours, reason _____
No ☐
Unknown ☐

Neonatal illness(es): Yes ☐ If YES, please state which illness(es) No ☐

_____

Neonatal abnormalities: Yes ☐ If YES, please state No ☐

_____

Feeding: Breast ☐ for __ __ weeks Bottle ☐ Mixed ☐

APPENDIX 3 - continued

Royal Free and University College Medical School
UNIVERSITY COLLEGE LONDON
Department of Community Child Health
Royal Free Campus
Rowland Hill Street
London NW3 2PF
Tel/Fax

Head of Department: Professor Brent Taylor *PhD, MB, ChB, FRCP, FRACP*
Lecturer in Child Health: Alastair Sutcliffe *MD, MRCP, MRCPCH*
Research Psychologist:: Xenya Chrysostomou *BSc Hons.*
Research Fellow: Catherine Peters *MBChB, MRCP,MRCPCH*

Patient Information **Date:** __ __ / __ __ / __ __

(f) Child Information

Identifier: _____ Name of child: _____

Age at evaluation: __ years, __ __ months Age today: __ years, __ _ months

Has your child had any illness(es)? Yes ☐ No ☐

If YES, please state _____

Has your child had any surgery? Yes ☐ No ☐

If YES, please state _____

No. of (in – patient) hospital admissions: __ __

Does your child take any medication(s)? Yes ☐ No ☐

If YES, please state _____

Does your child have any other therapy? Yes ☐ No ☐

If YES, please state _____

Measurements (percentiles to be calculated)

Length: _____cm Weight: _____ kg Head circumference: _____cm

Malformations (state whether they are major or minor)_____

Lung examination

Auscultation: Normal ☐ Abnormal ☐ Any comments _____

Heart examination

Heartmurmur: _____/6 Femoral pulses: Yes ☐ No ☐

APPENDIX 3 - continued

Royal Free and University College Medical School
UNIVERSITY COLLEGE LONDON
Department of Community Child Health
Royal Free Campus
Rowland Hill Street
London NW3 2PF
Tel/Fax 0171 830 2049 (**direct**) 0171 830 2440 (**dept.**) E. mail icsi@rfc.ucl.ac.uk

Head of Department: Professor Brent Taylor *PhD, MB, ChB, FRCP, FRACP*
Lecturer in Child Health: Alastair Sutcliffe *MD, MRCP, MRCPCH*
Research Psychologist:: Xenya Chrysostomou *BSc Hons.*
Research Fellow: Catherine Peters *MBChB, MRCP,MRCPCH*

Patient Information **Date:** __ __ / __ __ / __ __

(f) Child Information continued.....

Identifier: _____ Name of child: _____

Facial examination

Ears:	Normal	Abnormal	Unknown
Dentition:	Normal	Abnormal	Unknown
Nose:	Normal	Abnormal	Unknown
Adenopathy:	Normal	Abnormal	Unknown
Mouth:	Normal	Abnormal	Unknown

Further comments _____

_____

Abdomen

Liver: _____

Spleen: _____

Palpitation: _____

Genital examination

Boys

Normal □

Abnormal □ (please state) _____

Girls

Normal □ Abnormal □ (please state) _____

APPENDIX 3 - continued

Royal Free and University College Medical School
UNIVERSITY COLLEGE LONDON
Department of Community Child Health
Royal Free Campus
Rowland Hill Street
London NW3 2PF
Tel/Fax

Head of Department: Professor Brent Taylor *PhD, MB, ChB, FRCP, FRACP*
Lecturer in Child Health: Alastair Sutcliffe *MD, MRCP, MRCPCH*
Research Psychologist:: Xenya Chrysostomou *BSc Hons.*
Research Fellow: Catherine Peters *MBChB, MRCP,MRCPCH*

Patient Information **Date:** __ __ / __ __ / __ __

(f) Child Information continued.....

Identifier: _____ Name of child: _____

Dermatological examination

Skin:	Normal	Birthmark	Eczema
Hair:	Normal	Absent	Congenital abnormality
Nails:	Normal	Hypoplastic	Congenital abnormality
Skeleton:	Normal	Abnormal	_____

Neurological examination (comments)

Walk:	Normal	Abnormal	Cerebral Palsy
Other motor disorder	_____		
Jump:	Normal	Abnormal	Unknown
Run:	Normal	Abnormal	Unknown
Hopping 1 leg:	Normal	Abnormal	Unknown
Tonus:	Normal	Hypo	Hyper

Abnormalities _____

Audiometry

Left::	Normal	Abnormal	Collaboration	Difficult to interpret	Not done
Right:	Normal	Abnormal	Collaboration	Difficult to interpret	Not done

Comment_____

Ophthalmology

Cover test:	Normal	Abnormal			
Motility:	Normal	Abnormal			
Stereo:	Normal	Abnormal			
Snellen chart result:	__/ 60	Microstrabismus:	Yes	No	Not done

APPENDIX 4 - Data collection on sperm of ICSI fathers

Royal Free and University College Medical School
UNIVERSITY COLLEGE LONDON
Department of Community Child Health
Royal Free Campus
Rowland Hill Street
London NW3 2PF
Tel/Fax

Head of Department: Professor Brent Taylor *PhD, MB, ChB, FRCP, FRACP*
Lecturer in Child Health: Alastair Sutcliffe *MD, MRCP, MRCPCH*
Research Psychologist:: Xenya Chrysostomou *BSc Hons.*
Research Fellow: Catherine Peters *MBChB, MRCP,MRCPCH*

Patient Information **Date:** __ __ / __ __ / __ __

Data collection on ICSI/IVF* fathers (*delete as appropriate)

Identifier: _____ Name of child: _____

(a) Age: __ __

(b) Previous pregnancies: __ __

(c) Sperm source for treatment:

- ejaculate ☐
- testis ☐
- epididymis ☐
- bladder (retrograde) ☐

(d) Sperm for treatment:

- Fresh ☐
- Frozen ☐

(e) Karyotype:

- normal ☐
- abnormal ☐
- not available ☐

(f) Testicular biopsy:

- normal spermatogenesis ☐
- maturation arrest ☐
- Sertoli Only Syndrome ☐

(g) FSH: _____

Cystic Fibrosis carrier status:

- Negative ☐
- positive ☐ please describe _____
- not available ☐

APPENDIX 4 - continued

Royal Free and University College Medical School
UNIVERSITY COLLEGE LONDON
Department of Community Child Health
Royal Free Campus
Rowland Hill Street
London NW3 2PF
Tel/Fax

Head of Department: Professor Brent Taylor *PhD, MB, ChB, FRCP, FRACP*
Lecturer in Child Health: Alastair Sutcliffe *MD, MRCP, MRCPCH*
Research Psychologist:: Xenya Chrysostomou *BSc Hons.*
Research Fellow: Catherine Peters *MBChB, MRCP,MRCPCH*

Patient Information **Date:** __ __ / __ __ / __ __

Data collection on ICSI/IVF* fathers (*delete as appropriate)

Identifier: _____ Name of child: _____

If ejaculated sperm used, state parameters on the day:

Parameter	Fresh
Volume ____ ml	
Count _____ millions	
Motility __ __/__ __/__ __/__ __	
Morphology (standard method) __ __ %	
Morphology (Kruger strict criteria) __ __ %	

DIAGNOSTIC CATEGORY

(j) SURGICAL RECOVERY

Post vasectomy ☐
Congenital absence of vas ☐
Other obstructive ☐
Other ☐ (please state) _____
Testicular failure ☐
Anejaculation ☐
Psychosexual ☐

EJACULATED SPERM (may be a combination)

Post vasectomy reversal ☐
Oligozoospermia ☐
Asthenozoospermia ☐
Teratozoospermia ☐
Failed fertilisation with IVF ☐
Antisperm antibodies ☐

APPENDIX 5 - Parental questionnaires to assess family functioning

The questionnaires used to assess family functioning are included in this appendix. They are as follows:

8.1 Child Behaviour Checklist Questionnaire
8.2 Carey temperament Scales questionnaire
8.3 Parenting Stress Index Questionnaire
8.4 Parent Acceptance and Rejection Questionnaire
8.5 Greenberger Scales for Parental Commitment to Work and Parenting
8.6 Dyadic Adjustment Score Questionnaire
8.7 General Health Questionnaire

CHILD BEHAVIOR CHECKLIST FOR AGES 4-16

For office use only
ID #

by / in	AGE	RACE
ATE	CHILD'S BIRTHDATE	
_Day _____ Yr. _____	Mo. _____ Day _____ Yr. _____	

PARENT'S TYPE OF WORK *(Please be specific—for example: auto mechanic, high school teacher, homemaker, laborer, lathe operator, shoe salesman, army sergeant, even if parent does not live with child)*

FATHER'S TYPE OF WORK: _____

MOTHER'S TYPE OF WORK: _____

THIS FORM FILLED OUT BY:

- ☐ Mother
- ☐ Father
- ☐ Other *(Specify)*

e list the sports your child most likes to part in. For example: swimming, ball, skating, skate boarding, bike), fishing, etc.

☐ None

Compared to other children of the same age, about how much time does he/she spend in each?

	Don't Know	Less Than Average	Average	More Than Average
a. _____	☐	☐	☐	☐
b. _____	☐	☐	☐	☐
c. _____	☐	☐	☐	☐

Compared to other children of the same age, how well does he/she do each one?

	Don't Know	Below Average	Average	Above Average
a. _____	☐	☐	☐	☐
b. _____	☐	☐	☐	☐
c. _____	☐	☐	☐	☐

e list your child's favorite hobbies, ities, and games, other than sports. xample: stamps, dolls, books, piano, , singing, etc. (Do not include T.V.)

☐ None

Compared to other children of the same age, about how much time does he/she spend in each?

	Don't Know	Less Than Average	Average	More Than Average
a. _____	☐	☐	☐	☐
b. _____	☐	☐	☐	☐
c. _____	☐	☐	☐	☐

Compared to other children of the same age, how well does he/she do each one?

	Don't Know	Below Average	Average	Above Average
a. _____	☐	☐	☐	☐
b. _____	☐	☐	☐	☐
c. _____	☐	☐	☐	☐

e list any organizations, clubs, s, or groups your child belongs to.

☐ None

Compared to other children of the same age, how active is he/she in each?

	Don't Know	Less Active	Average	More Active
a. _____	☐	☐	☐	☐
b. _____	☐	☐	☐	☐
c. _____	☐	☐	☐	☐

e list any jobs or chores your child For example: paper route, babysitting, ing bed, etc.

☐ None

Compared to other children of the same age, how well does he/she carry them out?

	Don't Know	Below Average	Average	Above Average
a. _____	☐	☐	☐	☐
b. _____	☐	☐	☐	☐
c. _____	☐	☐	☐	☐

bout how many close friends does your child have? ☐ None ☐ 1 ☐ 2 or 3 ☐ 4 or more

bout how many times a week does your child do things with them? ☐ less than 1 ☐ 1 or 2 ☐ 3 or more

pared to other children of his/her age, how well does your child:

	Worse	About the same	Better
a. Get along with his/her brothers & sisters?	☐	☐	☐
b. Get along with other children?	☐	☐	☐
c. Behave with his/her parents?	☐	☐	☐
d. Play and work by himself/herself?	☐	☐	☐

urrent school performance—for children aged 6 and older:

☐ Does not go to school

	Failing	Below average	Average	Above average
a. Reading or English	☐	☐	☐	☐
b. Writing	☐	☐	☐	☐
c. Arithmetic or Math	☐	☐	☐	☐
d. Spelling	☐	☐	☐	☐
academic sub- e. _____	☐	☐	☐	☐
-for example: his- f. _____	☐	☐	☐	☐
cience, foreign g. _____	☐	☐	☐	☐
ge, geography.				

Is your child in a special class?

☐ No ☐ Yes—what kind?

Has your child ever repeated a grade?

☐ No ☐ Yes—grade and reason

Has your child had any academic or other problems in school?

☐ No ☐ Yes—please describe

When did these problems start?

Have these problems ended?

☐ No ☐ Yes—when?

ow is a list of items that describe children. For each item that describes your child now or within the past 6 months, please circle 2 if the item is very true or often true of your child. Circle the 1 if the item is somewhat or sometimes true of your child. If the item ot true of your child, circle the 0. Please answer all items as well as you can, even if some do not seem to apply to your child.

0 = Not True (as far as you know) 1 = Somewhat or Sometimes True 2 = Very True or Often True

	1.	Acts too young for his/her age	16	0 1 2	31.	Fears he/she might think or do something bad
	2.	Allergy (describe): _____		0 1 2	32.	Feels he/she has to be perfect
				0 1 2	33.	Feels or complains that no one loves him/her
	3.	Argues a lot		0 1 2	34.	Feels others are out to get him/her
	4.	Asthma		0 1 2	35.	Feels worthless or inferior 50
	5.	Behaves like opposite sex	20	0 1 2	36.	Gets hurt a lot, accident-prone
	6.	Bowel movements outside toilet		0 1 2	37.	Gets in many fights
	7.	Bragging, boasting		0 1 2	38.	Gets teased a lot
	8.	Can't concentrate, can't pay attention for long		0 1 2	39.	Hangs around with children who get in trouble
	9.	Can't get his/her mind off certain thoughts; obsessions (describe): _____		0 1 2	40.	Hears things that aren't there (describe): _____ 55
	10.	Can't sit still, restless, or hyperactive	25	0 1 2	41.	Impulsive or acts without thinking
	11.	Clings to adults or too dependent		0 1 2	42.	Likes to be alone
	12.	Complains of loneliness		0 1 2	43.	Lying or cheating
	13.	Confused or seems to be in a fog		0 1 2	44.	Bites fingernails
	14.	Cries a lot		0 1 2	45.	Nervous, highstrung, or tense 60
	15.	Cruel to animals	30	0 1 2	46.	Nervous movements or twitching (describe): _____
	16.	Cruelty, bullying, or meanness to others		0 1 2	47.	Nightmares
	17.	Day-dreams or gets lost in his/her thoughts		0 1 2	48.	Not liked by other children
	18.	Deliberately harms self or attempts suicide		0 1 2	49.	Constipated, doesn't move bowels
	19.	Demands a lot of attention		0 1 2	50.	Too fearful or anxious 65
	20.	Destroys his/her own things	35	0 1 2	51.	Feels dizzy
	21.	Destroys things belonging to his/her family or other children		0 1 2	52.	Feels too guilty
	22.	Disobedient at home		0 1 2	53.	Overeating
	23.	Disobedient at school		0 1 2	54.	Overtired
	24.	Doesn't eat well		0 1 2	55.	Overweight 70
	25.	Doesn't get along with other children	40		56.	Physical problems without known medical cause:
	26.	Doesn't seem to feel guilty after misbehaving		0 1 2		a. Aches or pains
	27.	Easily jealous		0 1 2		b. Headaches
	28.	Eats or drinks things that are not food (describe): _____		0 1 2		c. Nausea, feels sick
				0 1 2		d. Problems with eyes (describe): _____
	29.	Fears certain animals, situations, or places, other than school (describe): _____		0 1 2		e. Rashes or other skin problems 75
				0 1 2		f. Stomachaches or cramps
				0 1 2		g. Vomiting, throwing up
	30.	Fears going to school	45	0 1 2		h. Other (describe): _____

Please see other side

0 = Not True (as far as you know) 1 = Somewhat or Sometimes True 2 = Very True or Often True

2	57.	Physically attacks people	0	1	2	84.	Strange behavior (describe):
2	58.	Picks nose, skin, or other parts of body (describe):	0	1	2	85.	Strange ideas (describe):
2	59.	Plays with own sex parts in public	0	1	2	86.	Stubborn, sullen, or irritable
2	60.	Plays with own sex parts too much	0	1	2	87.	Sudden changes in mood or feelings
2	61.	Poor school work	0	1	2	88.	Sulks a lot
2	62.	Poorly coordinated or clumsy	0	1	2	89.	Suspicious
2	63.	Prefers playing with older children	0	1	2	90.	Swearing or obscene language
2	64.	Prefers playing with younger children	0	1	2	91.	Talks about killing self
2	65.	Refuses to talk	0	1	2	92.	Talks or walks in sleep (describe):
2	66.	Repeats certain acts over and over; compulsions (describe):	0	1	2	93.	Talks too much
2	67.	Runs away from home	0	1	2	94.	Teases a lot
2	68.	Screams a lot	0	1	2	95.	Temper tantrums or hot temper
2	69.	Secretive, keeps things to self	0	1	2	96.	Thinks about sex too much
2	70.	Sees things that aren't there (describe):	0	1	2	97.	Threatens people
2	71.	Self-conscious or easily embarrassed	0	1	2	98.	Thumb-sucking
2	72.	Sets fires	0	1	2	99.	Too concerned with neatness or cleanliness
2	73.	Sexual problems (describe):	0	1	2	100.	Trouble sleeping (describe):
2	74.	Showing off or clowning	0	1	2	101.	Truancy, skips school
2	75.	Shy or timid	0	1	2	102.	Underactive, slow moving, or lacks energy
2	76.	Sleeps less than most children	0	1	2	103.	Unhappy, sad, or depressed
2	77.	Sleeps more than most children during day and/or night (describe):	0	1	2	104.	Unusually loud
2	78.	Smears or plays with bowel movements	0	1	2	105.	Uses alcohol or drugs (describe):
2	79.	Speech problem (describe):	0	1	2	106.	Vandalism
2	80.	Stares blankly	0	1	2	107.	Wets self during the day
2	81.	Steals at home	0	1	2	108.	Wets the bed
2	82.	Steals outside the home	0	1	2	109.	Whining
2	83.	Stores up things he/she doesn't need (describe):	0	1	2	110.	Wishes to be of opposite sex
			0	1	2	111.	Withdrawn, doesn't get involved with others
			0	1	2	112.	Worrying
						113.	Please write in any problems your child has that were not listed above:

E BE SURE YOU HAVE ANSWERED ALL ITEMS.

UNDERLINE ANY YOU ARE CONCERNED ABOUT.

8.2 Parent Temperament Questionnaire For Children Aged 3 – 7 Years Of Age

This questionnaire is designed to gather information on the way your child behaves in different situations of everyday life. Each statement asks you to judge whether that behavior occurs hardly ever, infrequently, once in a while, sometimes, often, very often or almost always. After each statement, please circle the number from 1 to 7 that describes your child's behavior. The statements often involve making judgments (such as whether your child does something "quickly" or "slowly" for a "long time" and so on). Please try to make these judgments to the best of your ability based on how you think your child compares to other children of about the same age.

Some statements may seem similar to each other because they ask about the same situation. However, each one looks at a different area of the child's behaviour. Therefore, your answers may be different in each case. Should you feel that some of the choices you make need more explanation because you are uncertain about that particular choice or because you feel that your child's behavior in that area is special enough to call for more information, please circle the choice that seems to fit best and then write a brief note under "comments" at the end of the questionnaire. For example, if you feel that on some specific item your child's behavior "never" occurs or "always" occurs" circle the "almost always" or "hardly ever" and indicate that it is "always" or "never" in the comment.

A few items may not apply to your child (such as questions about school for those children not yet at school). In that case please write "NA" (not applicable) next to the item.

1	2	3	4	5	6	7
hardly ever	**infrequently**	**once in a while**	**sometimes**	**often**	**very often**	**almost always**

1. My child splashes hard in the bath and plays actively.	hardly ever	1	2	3	4	5	6	7	almost always
2. When with other children, my child seems to be having a good time.	hardly ever	1	2	3	4	5	6	7	almost always
3. My child quickly notices odours and comments on unpleasant smells.	hardly ever	1	2	3	4	5	6	7	almost always
4. My child is shy with adults he/she does not know.	hardly ever	1	2	3	4	5	6	7	almost always
5. When my child starts a project such as a model, puzzle, painting, he/she works at it without stopping until completed even if it takes a long time.	hardly ever	1	2	3	4	5	6	7	almost always
6. My child has a bowel movement at the same time every day.	hardly ever	1	2	3	4	5	6	7	almost always

7. My child now eats food that he/she used to dislike.	hardly ever	1	2	3	4	5	6	7	almost always
8. My child shows strong enthusiasm for food he/she likes or strong dislike for food he/she does not like.	hardly ever	1	2	3	4	5	6	7	almost always
9. If my child is in a bad mood he/she can easily be "joked" out of it.	hardly ever	1	2	3	4	5	6	7	almost always
10. When first meeting new children my child is bashful.	hardly ever	1	2	3	4	5	6	7	almost always
11. My child ignores loud noises. For example he/she is the last to complain about music being too loud, sirens etc.	hardly ever	1	2	3	4	5	6	7	almost always
12. If my child is not permitted to wear an item of clothing he/she accepts wearing mother's choice after a short discussion.	hardly ever	1	2	3	4	5	6	7	almost always
13. My child asks for or takes a Snack at approximately the same time every day.	hardly ever	1	2	3	4	5	6	7	almost always
14. My child is happy and pleased when telling about something that has happened during the day.	hardly ever	1	2	3	4	5	6	7	almost always
15. My child is at ease within a few visits when visiting at someone else's home.	hardly ever	1	2	3	4	5	6	7	almost always
16. When upset or annoyed with a task my child may throw it down, cry, yell or slam door etc.	hardly ever	1	2	3	4	5	6	7	almost always
17. If my child wants a toy or candy (while shopping) he/ she will easily accept something else. offered instead	hardly ever	1	2	3	4	5	6	7	almost always
18. When my child moves about in a house or outdoors he/she runs rather than walks.	hardly ever	1	2	3	4	5	6	7	almost always
19. My child enjoys going shopping with parents.	hardly ever	1	2	3	4	5	6	7	almost always
20. After my child is put to bed at night it takes about the same length of time to fall asleep.	hardly ever	1	2	3	4	5	6	7	almost always

21. My child likes to try new foods.	hardly ever	1	2	3	4	5	6	7	almost always
22. When mother is busy and cannot do what child wants, he/she goes away and does something else instead of keeping after mother.	hardly ever	1	2	3	4	5	6	7	almost always
23. My child quickly notices colours e.g.may comment on how pretty or ugly they are.	hardly ever	1	2	3	4	5	6	7	almost always
24. In the playground, my child runs, climbs, swings and is consistently on the go.	hardly ever	1	2	3	4	5	6	7	almost always
25. If my child resists some procedure such as having hair cut, brushed or washed, he/she will continue to resist it for at least several months.	hardly ever	1	2	3	4	5	6	7	almost always
26. If there is a sudden noise or activity nearby when my child is playing with a favourite toy he/she ignores it or at most looks up briefly.	hardly ever	1	2	3	4	5	6	7	almost always
27. When taken away from an activity that my child really enjoys he/she protests only mildly with a little bit of fussing or some whining.	hardly ever	1	2	3	4	5	6	7	almost always
28. When my child is promised something in the future he/she keeps reminding parents consistently.	hardly ever	1	2	3	4	5	6	7	almost always
29. When playing with other children, my child argues with them.	hardly ever	1	2	3	4	5	6	7	almost always
30. When in the park or at a party my child will go up to strange children and join in their play.	hardly ever	1	2	3	4	5	6	7	almost always
31. My child sleeps more one night and less another night rather than the same number of hours each night.	hardly ever	1	2	3	4	5	6	7	almost always

32. My child ignores the temperature of foods (hot or cold).	hardly ever	1	2	3	4	5	6	7	almost always
33. If my child is shy with a strange adult he/she quickly (within a half hour or so) gets over this.	hardly ever	1	2	3	4	5	6	7	almost always
34. My child insists still to have a story told or read or a song sung.	hardly ever	1	2	3	4	5	6	7	almost always
35. When scolded or reprimanded by parents my child reacts mildly such as whining or complaining rather than strongly with crying or screaming.	hardly ever	1	2	3	4	5	6	7	almost always
36. When my child becomes angry about something it is difficult to sidetrack him/her.	hardly ever	1	2	3	4	5	6	7	almost always
37. When learning a new physical activity (such as hoping, skating,bike riding) my child will spend long periods of time practicing.	hardly ever	1	2	3	4	5	6	7	almost always
38. My child gets hungry at different times each day.	hardly ever	1	2	3	4	5	6	7	almost always
39. My child is highly sensitive to changes in the brightness or dimness of light.	hardly ever	1	2	3	4	5	6	7	almost always
40. When away from home with parents my child has a problem (even after a few nights) in falling asleep in a new bed.	hardly ever	1	2	3	4	5	6	7	almost always
41. My child looks forward to going to school.	hardly ever	1	2	3	4	5	6	7	almost always
42. When the family takes a trip, my child immediately makes self at home in the new surroundings.	hardly ever	1	2	3	4	5	6	7	almost always
43. When shopping together and mother does not buy candy, toys or clothingthat child wants he/she cries and yells.	hardly ever	1	2	3	4	5	6	7	almost always
44. If my child is upset it is hard to comfort him/her.	hardly ever	1	2	3	4	5	6	7	almost always

45. When the weather is bad and my child is confined to the house he/she runs around and cannot be entertained by quiet activities.	hardly ever	1	2	3	4	5	6	7	almost always
46. My child is immediately friendly with and approaches unknown adults who visit our home.	hardly ever	1	2	3	4	5	6	7	almost always
47. My child eats a lot one day and very little the next day rather than the same amount each day.	hardly ever	1	2	3	4	5	6	7	almost always
48. When a toy or game is difficult my child will turn quickly to another activity.	hardly ever	1	2	3	4	5	6	7	almost always
49. My child ignores differences in temperature indoors or outdoors.	hardly ever	1	2	3	4	5	6	7	almost always
50. If a favourite toy or game is broken my child gets noticeably upset.	hardly ever	1	2	3	4	5	6	7	almost always
51. In a new situation such as a nursery,day care centre or school my child is still uncomfortable even after a few days.	hardly ever	1	2	3	4	5	6	7	almost always
52. Although my child dislikes some procedures (such as nail cutting or hair brushing), he/she will easily allow it if watching television or being entertained while it is done.	hardly ever	1	2	3	4	5	6	7	almost always
53. My child can sit quietly through an entire children's movie, game or a long TV programme.	hardly ever	1	2	3	4	5	6	7	almost always
54. When my child objects to wearing certain clothing he/she argues loudly, yells, cries.	hardly ever	1	2	3	4	5	6	7	almost always
55. On weekends and holidays my child wakes himself/ herself up at the sametime each morning.	hardly ever	1	2	3	4	5	6	7	almost always
56. My child complains to own parents about other children if anything goes wrong.	hardly ever	1	2	3	4	5	6	7	almost always

57. My child is sensitive and complains about clothing being tight, itchy or uncomfortable.	hardly ever	1	2	3	4	5	6	7	almost always
58. If my child is angry or annoyed he/she gets over it quickly.	hardly ever	1	2	3	4	5	6	7	almost always
59. When there is a change in daily routine such as not being ale to go to school change of usual daily activities etc. my child goes along with the new routine easily.	hardly ever	1	2	3	4	5	6	7	almost always
60. When outdoors in a playground or park my child plays quietly with toys or dolls.	hardly ever	1	2	3	4	5	6	7	almost always
61. My child complains quietly when another child takes his/her toy away.	hardly ever	1	2	3	4	5	6	7	almost always
62. The first time my child is left in a new situation without mother (such as school, nursery, music lesson) he/she gets upset.	hardly ever	1	2	3	4	5	6	7	almost always
63. If my child starts to play with something and I want them to stop it is hard to turn their attention to something else.	hardly ever	1	2	3	4	5	6	7	almost always
64. My child gets involved in quiet activities such as crafts, watching television, reading or looking at picture books.	hardly ever	1	2	3	4	5	6	7	almost always
65. My child becomes easily upset when he/she loses a game.	hardly ever	1	2	3	4	5	6	7	almost always
66. My child would rather wear familiar clothes than new clothes.	hardly ever	1	2	3	4	5	6	7	almost always
67. If my child gets dirty or wet, he/she ignores this and appears quite comfortable.	hardly ever	1	2	3	4	5	6	7	almost always
68. My child has difficulty in adjusting to rules of another household if they are different from those at home.	hardly ever	1	2	3	4	5	6	7	almost always

69. My child seems to take things matter of factly. Accepts events in stride without getting very excited.	hardly ever	1	2	3	4	5	6	7	almost always
70. If meals are delayed for a hour or more my child easily waits without seeming to mind.	hardly ever	1	2	3	4	5	6	7	almost always
71. My child can be stopped from pestering if he/she is given something else to do.	hardly ever	1	2	3	4	5	6	7	almost always
72. When assistance is offered in doing a task, my child continues to do it on his/her own.	hardly ever	1	2	3	4	5	6	7	almost always

Comments:

8.3 The Parenting Stress Index

Name _____

Gender _____ **Date of Birth** __ __/__ __/__ __

Ethnic Group _____ **Marital Status** _____

Child's Name _____ **Child's Gender** _____

Child's Date of Birth __ __/ __ __/ __ __ **Today's Date** __ __/ __ __/ __ __

SA = Strongly Agree A = Agree NS = Not Sure D = Disagree SD = Strongly Disagree

Please write your answer next to each statement.

1. I often have the feeling that I cannot handle things very well.
2. I find myself giving up more of my life to meet my children's needs that I ever expected.
3. I feel trapped by my responsibilities as a parent.
4. Since having this child, I have been unable to do new and different things.
5. Since having this child, I feel that I am almost never able to do things that I like to do.
6. I am unhappy with the last purchase of clothing I made for myself.
7. There are quite a few things that bother me about my life.
8. Having a child has caused more problems than I expected in my relationship with my spouse (male/female friend).
9. I feel along and without friends.
10. When I go to a party I usually expect not to enjoy myself.
11. I am not as interested in people as I used to be.
12. I don't enjoy things as I used to.
13. My child rarely does things for me that make me feel good.
14. Most times I feel that my child does not like me and does not want to be close to me.
15. My child smiles at me much less than I expected.
16. When I do things for my child, I get the feeling that my efforts are not appreciated very much.
17. When playing, my child doesn't often giggle or laugh.
18. My child doesn't seem to learn as quickly as most children.
19. My child doesn't seem to smile as much as most children.
20. My child is not able to do as much as I expected.
21. It takes a long time and it is very hard for my child to get used to new things.

For the next statement, choose your response from the choices 1 to 5 below.

22. I feel that I am:
    1. not very good at being a parent
    2. a person who has some trouble beings parent
    3. an average parent
    4. a better than average parent
    5. a very good parent

23. I expected to have closer and warmer feeling for my child than I do - this bothers me
24. Sometimes my child does things that other me just to be mean.
25. My child seem sot cry or fuss more often that most other children.
26. My child generally wakes up in a bad mood.
27. I feel that my child is very moody and easily upset.
28. My child does few things that bother me a great deal.
29. My child reacts very strongly when something happens that my child doesn't like.
30. My child gets upset easily over the smallest thing.
31. My child's sleeping or eating schedule was much harder to establish that I expected.

For the next statement, circle your response from the choices 1 to 5 below.

32. I have found that getting my child to do something or stop doing something is:
    1. much harder than I expected
    2. somewhat harder than I expected
    3. about as hard as I expected
    4. somewhat harder than I expected
    5. much easier than I expected

For the next statement, circle your response from the choices 10+ to 1-3.

33. Think carefully and count the number of things that your child does that bother you. For example: dawdles, refuses to listen, overactive, cries, interrupts, fights, whines, etc.

10+ 8-9 6-7 4-5 1-3

34. There are some things my child does that really bother me a lot.
35. My child turned out to be more of a problem that I had expected.
36. My child makes more demands on me than most children.

Thank you for taking the time to complete this questionnaire.

8.4 Parental Acceptance and rejection questionnaire

Name of child _____ Name of Parent _____
Date: __ __/ __ __/ __ __

The following pages contain a number of statements describing the way different parents act toward their children. Read each statement carefully and think how well it describes the way you treat your child. Work quickly, give your first impression and move on to the next item. Do not dwell on any item.

Four lines are drawn after each sentence. If the statement is basically true about the way you treat your child then ask yourself. "Is it almost always true?" or "Is it only sometimes true?" If you think you almost always treat your child that way, put an X on the line ALMOST ALWAYS TRUE; if the statement is sometimes true about the way you treat your child, then mark SOMETIMES TRUE. If you feel the statement is basically untrue about the way you treat your child then ask yourself, "Is it rarely true?" or "Is it almost never true? If it is rarely true about the way you treat your child put an X on the line RARELY TRUE; if you feel the statement is almost never true then mark ALMOST NEVER TRUE.

Remember, there is no right or wrong answer to any statement so be as frank as you can. Respond to each statement the way you feel you really are rather than the way you might like to be. For example, if you almost always hug and kiss your child when he/she is good, you could mark the item as follows:

	TRUE OF ME		NOT TRUE OF ME	
	Almost Always True	Sometimes True	Rarely True	Almost Never True
1. I hug and kiss my child when he/she is good	__ X __	_____	_____	_____

	TRUE OF ME		NOT TRUE OF ME	
	Almost Always True	Sometimes True	Rarely True	Almost Never True
1. I say nice things about my child	_____	_____	_____	_____
2. I nag or scold my child when he/she is bad.	_____	_____	_____	_____
3. I ignore my child.	_____	_____	_____	_____
4. I wonder if I really love my child.	_____	_____	_____	_____
5. I discuss general daily routines with my child and listen to what he/she has to say.	_____	_____	_____	_____
6. I complain about my child to others when he/she does not listen to me.	_____	_____	_____	_____
7. I take an active interest in my child.	_____	_____	_____	_____
8. I encourage my child to bring friends home and I try to make things pleasant for them.	_____	_____	_____	_____
9. I make fun of my child.	_____	_____	_____	_____
10. I ignore my child as long as he/she does not do anything to disturb me.	_____	_____	_____	_____
11. I yell at my child when I am angry	_____	_____	_____	_____
12. I make it easy for my child to confide in me.	_____	_____	_____	_____
13. I am harsh with my child.	_____	_____	_____	_____
14. I enjoy having my child around me.	_____	_____	_____	_____
15. I make my child feel proud when he/she does well.	_____	_____	_____	_____
16. I hit my child even when he/she may not deserve it.	_____	_____	_____	_____

	TRUE OF ME		NOT TRUE OF ME	
	Almost Always True	Sometimes True	Rarely True	Almost Never True
17. I forget things I am supposed to do for my child.	_____	_____	_____	_____
18. My child is a burden for me.	_____	_____	_____	_____
19. I praise my child to others.	_____	_____	_____	_____
20. I punish my child when I am angry.	_____	_____	_____	_____
21. I make sure my child has the right kind of food to eat.	_____	_____	_____	_____
22. I talk to my child in a warm and affectionate way.	_____	_____	_____	_____
21. I am impatient with my child.	_____	_____	_____	_____
24. I am too busy to answer my child's questions.	_____	_____	_____	_____
25. I resent my child.	_____	_____	_____	_____
26. I praise my child when he/she deserves it.	_____	_____	_____	_____
27. I am irritable with my child.	_____	_____	_____	_____
28. I am concerned who my child's friends are.	_____	_____	_____	_____
29. I take real interest in my child's affairs.	_____	_____	_____	_____
30. I say unkind things to my child.	_____	_____	_____	_____
31. I ignore my child when he/she asks for help.	_____	_____	_____	_____
32. I am unsympathetic to my child when he/she is having trouble.	_____	_____	_____	_____

	TRUE OF ME		NOT TRUE OF ME	
	Almost Always True	Sometimes True	Rarely True	Almost Never True
33. I make my child feel wanted and needed.	_____	_____	_____	_____
34. I tell my child that he/she gets on my nerves.	_____	_____	_____	_____
35. I pay a lot of attention to my child.	_____	_____	_____	_____
36. I tell my child how proud I am of him/her when he/she is good.	_____	_____	_____	_____
37. I hurt my child's feelings.	_____	_____	_____	_____
38. I forget events that my child thinks I should remember.	_____	_____	_____	_____
39. When my child misbehaves, I make him/her feel I don't love him/her anymore.	_____	_____	_____	_____
40. I make my child feel what he/she does is important.	_____	_____	_____	_____
41. When my child does something wrong, I threaten or frighten him/her.	_____	_____	_____	_____
42. I like to spend time with my child.	_____	_____	_____	_____
43. I try to help my child when he/she is scared or upset.	_____	_____	_____	_____
44. When my child misbehaves, I shame him/her in front of his/her playmates.	_____	_____	_____	_____
45. I avoid my child's company.	_____	_____	_____	_____
46. I complain about my child.	_____	_____	_____	_____
47. I respect my child's point of view and encourage him/her to express it.	_____	_____	_____	_____

	TRUE OF ME		NOT TRUE OF ME	
	Almost Always True	Sometimes True	Rarely True	Almost Never True
48. I compare my child unfavourably with other children.	_____	_____	_____	_____
49. When I make plans, I take my child into considerations.	_____	_____	_____	_____
50. I let my child do things he/she thinks are important, even if it is inconvenient for me.	_____	_____	_____	_____
51. When my child misbehaves, I compare him/her unfavourably with other children.	_____	_____	_____	_____
52. I leave my child to someone else's care e.g. neighbour or relative.	_____	_____	_____	_____
53. I let my child know he/she is not wanted.	_____	_____	_____	_____
54. I am interested in the things my child does.	_____	_____	_____	_____
55. I try to make my child feel better.	_____	_____	_____	_____
56. I tell my child I am ashamed of him/her when he/she misbehaves.	_____	_____	_____	_____
57. I let my child know I love him/her.	_____	_____	_____	_____
58. I treat my child gently and kindly.	_____	_____	_____	_____
59. When my child misbehaves, I make him/her feel ashamed or guilty.	_____	_____	_____	_____
60. I try to make my child happy.	_____	_____	_____	_____

8.5 Greenberger Parenting and Work Commitment

YOU AND YOUR WORK

Using the options listed below, please indicate how strongly you agree or disagree with each of the following statements by putting the option number next to each statement.

1	2	3	4	5	6
Strongly disagree	**Disagree**	**Slightly disagree**	**Slightly agree**	**Agree**	**Strongly agree**

1. When I meet people, one of the first things I tell them about myself is the sort of work I do.
2. I often find myself thinking about some aspect of my job during non – work hours.
3. I want to advance to the top in my career, even if it involves some costs in other areas of my life.
4. For me it is more important to help my spouse in his/her career than to advance to the top in my own career.
5. I am more likely to talk to my friends about my spouse's work than about my own work.
6. I can't picture having a fully satisfying life without a career.
7. I would continue to work even if I didn't need the income.
8. I want to have more and more authority and responsibility in my job as time goes on.
9. The work I do is extremely interesting to me.
10. I often spend my so – called "free – time" working.
11. I don't think I was really cut out to work all my life.
12. I find that I put work responsibilities ahead of family responsibilities.
13. When I'm out for an evening, I don't enjoy talking about my work.
14. How I'm doing in my job is central to my self – esteem.
15. When I have time alone with my spouse I like to talk about my work.
16. I give up personal pleasures such as extra sleep or doing tins for fun to spend time on my work.
17. I often put in extra hours at my job either working over – time or doing extra work on my own.

ABOUT BEING A PARENT

Please indicate, by circling an option, to what extent you agree or disagree with the following questions.

IMPORTANT: When the phrases "my child" is used, please focus on the preschool child you have been describing.

1. On week nights, I'm usually too busy or tired to play enthusiastically with my child.

1	2	3	4	5	6
AGREE very strongly	**AGREE strongly**	**AGREE slightly**	**DISAGREE slightly**	**DISAGREE strongly**	**DISAGREE very strongly**

2. I often find myself thinking about my child when I'm at work.

1	2	3	4	5	6
AGREE very strongly	**AGREE strongly**	**AGREE slightly**	**DISAGREE slightly**	**DISAGREE strongly**	**DISAGREE very strongly**

3. I probably talk too much about my child.

1	2	3	4	5	6
AGREE very strongly	**AGREE strongly**	**AGREE slightly**	**DISAGREE slightly**	**DISAGREE strongly**	**DISAGREE very strongly**

4. I do not take my child to "child events" unless I expect to enjoy them too.

1	2	3	4	5	6
AGREE very strongly	**AGREE strongly**	**AGREE slightly**	**DISAGREE slightly**	**DISAGREE strongly**	**DISAGREE very strongly**

5. I seriously wonder whether I was cut out to be a parent.

1	2	3	4	5	6
AGREE very strongly	**AGREE strongly**	**AGREE slightly**	**DISAGREE slightly**	**DISAGREE strongly**	**DISAGREE very strongly**

6. When I meet new people, one of the first things I ell them about is my child.

1	2	3	4	5	6
AGREE very strongly	**AGREE strongly**	**AGREE slightly**	**DISAGREE slightly**	**DISAGREE strongly**	**DISAGREE very strongly**

7. I would not be ale to lower my career goals in order to spend more time with my child.

1	2	3	4	5	6
AGREE very strongly	**AGREE strongly**	**AGREE slightly**	**DISAGREE slightly**	**DISAGREE strongly**	**DISAGREE very strongly**

8. I don' like to talk about children when I'm out for an evening.

1	2	3	4	5	6
AGREE very strongly	**AGREE strongly**	**AGREE slightly**	**DISAGREE slightly**	**DISAGREE strongly**	**DISAGREE very strongly**

9. I ask my child for a lot of details about what he or she did during the day.

1	2	3	4	5	6
AGREE very strongly	**AGREE strongly**	**AGREE slightly**	**DISAGREE slightly**	**DISAGREE strongly**	**DISAGREE very strongly**

10. Being a parent allows me to express some of the traits and values I most prize in myself.

1	2	3	4	5	6
AGREE very strongly	**AGREE strongly**	**AGREE slightly**	**DISAGREE slightly**	**DISAGREE strongly**	**DISAGREE very strongly**

11. Being a parent is important to me but isn't central in how I define myself.

1	2	3	4	5	6
AGREE very strongly	**AGREE strongly**	**AGREE slightly**	**DISAGREE slightly**	**DISAGREE strongly**	**DISAGREE very strongly**

12. It's enough to be a good parent, I don't expect myself to be a model parent.

1	2	3	4	5	6
AGREE very strongly	**AGREE strongly**	**AGREE slightly**	**DISAGREE slightly**	**DISAGREE strongly**	**DISAGREE very strongly**

13. I give up personal pleasures such as extra sleep or socialising with friends to be with my child.

1	2	3	4	5	6
AGREE very strongly	**AGREE strongly**	**AGREE slightly**	**DISAGREE slightly**	**DISAGREE strongly**	**DISAGREE very strongly**

14. Children seem to grow like weeds. They don't need a great deal of "working on" by their parents.

1	2	3	4	5	6
AGREE very strongly	**AGREE strongly**	**AGREE slightly**	**DISAGREE slightly**	**DISAGREE strongly**	**DISAGREE very strongly**

15. I can not imagine a studying life with children.

1	2	3	4	5	6
AGREE very strongly	**AGREE strongly**	**AGREE slightly**	**DISAGREE slightly**	**DISAGREE strongly**	**DISAGREE very strongly**

16. I can't concentrate on my work if my child is ill.

1	2	3	4	5	6
AGREE very strongly	**AGREE strongly**	**AGREE slightly**	**DISAGREE slightly**	**DISAGREE strongly**	**DISAGREE very strongly**

17. Being a parent isn't as rewarding as I had expected it to be.

1	2	3	4	5	6
AGREE very strongly	**AGREE strongly**	**AGREE slightly**	**DISAGREE slightly**	**DISAGREE strongly**	**DISAGREE very strongly**

8.6 The Dyadic Adjustment Scale

Name: _____ Child's Name: _____

Most persons have disagreements in their relationship(s). Please indicate below the approximate extent of agreement or disagreement between you and your partner for each item on the following list. (Could you kindly place a tick, √, to indicate your answer).

	Always Agree	Almost Always Agree	Occasionally Disagree	Frequently Disagree	Almost Always Disagree	Always Disagree
1. Handling family finances	____	____	____	____	____	____
2. Matters of recreation	____	____	____	____	____	____
3. Religious matters	____	____	____	____	____	____
4. Demonstration of Affection	____	____	____	____	____	____
5. Friends	____	____	____	____	____	____
6. Sex relations	____	____	____	____	____	____
7. Conventionality (correct or proper behaviour)	____	____	____	____	____	____
8. Philosophy of life	____	____	____	____	____	____
9. Ways of dealing with parents or in – laws	____	____	____	____	____	____
10. Aims, goals and things believed important	____	____	____	____	____	____
11. Amount of time spent together	____	____	____	____	____	____
12. Making major decisions	____	____	____	____	____	____
13. Household tasks	____	____	____	____	____	____
14. Leisure time interests and activities	____	____	____	____	____	___
15. Career decisions	____	____	____	____	____	____

	Never	All the time	Most of the time	More often than not	Occasionally	Rarely
16. How often do you discuss or have you considered divorce, separation or terminating your relationship?	____	____	____	____	____	____
17. How often do you or your partner leave the house after a fight?	____	____	____	____	____	____
18. In general, how often do you think that things between you and your partner are going well?	____	____	____	____	____	____
19. Do you confide in your partner?	____	____	____	____	____	____
20. Do you ever regret that you married/live together/ began the relationship?	____	____	____	____	____	____
21. How often do you and your partner quarrel?	____	____	____	____	____	____
22. How often do you and your partner "get on each other's nerves"?	____	____	____	____	____	____

	Every Day	Almost Every Day	Occasionally	Rarely	Never
23. Do you kiss your partner?	____	____	____	____	____

	All of them	Most of them	Some of of them	Very few of them	None of them
24. Do you and your partner engage in outside interests together?	____	____	____	____	____

How often would you say the following events occur between you and your partner?

		Never	Less than once a month	Once or twice a month	Once or twice a week	Once a day	More often
25.	Have a stimulating exchange of ideas.	____	____	____	____	____	____
26.	Laugh together	____	____	____	____	____	____
27.	Calmly discuss something	____	____	____	____	____	____
28.	Work together on a project	____	____	____	____	____	____

There are some things about which couples sometimes agree and sometimes disagree. Indicate if either item below caused differences of opinions or were problems in your relationship during the past few weeks. (Tick yes or no).

	YES	NO	
29.	____	____	Being too tired for sex.
30.	____	____	Not showing love.

31. The dots on the following line represent different degrees of happiness in your relationship. The middle point, "happy", represents the degree of happiness of most relationships. Please circle the one which best describes the degree of happiness, all things considered, of your relationship.

•———•———•———•———•———•———•

Extremely unhappy | Fairly happy | A little unhappy | Happy | Very happy | Extremely happy | Perfect

32. Which of the following statements best describes how you feel about the future of your relationship?

____ I want desperately for my relationship to succeed and would go to almost any length to see that it does.

____ I want very much for my relationship to succeed and will do all I can to see that it does.

____ I want very much for my relationship to succeed and will do my fair share to see that it does.

____ It would be nice if my relationship succeeded but I can't do much more than I am doing now to help it succeed.

____ My relationship can never succeed and there is no more that I can do to keep the relationship going.

Thank you for taking the time to complete this questionnaire.

8.7 The General Health Questionnaire

Name: _____ Child's Name: _____

Please read this carefully.

We would like to know if you have had any medical complaints and how your health has been in general, **over the past few weeks.** Please answer ALL questions on the following pages by circling the answer, which you think most nearly, applies to you. Remember that we want to know about present and recent complaints, not those that you have had in the past. It is important that you try to answer ALL the questions. Thank you very much for your co - operation.

HAVE YOU RECENTLY:

A1.	Been feeling perfectly well and in good health?	Better than usual	Same as usual	Worse than usual	Much worse than usual
A2.	Been feeling in need of a good tonic?	Not at all	No more than usual	Rather more than usual	Much more than usual
A3.	Been feeling run down and out of sorts?	Not at all usual	No more than than usual	Rather more than usual	Much more than usual
A4.	Felt that you are ill?	Not at all	No more than usual	Rather more than usual	Much more than usual
A5.	Been getting pains in your head?	Not at all	No more than usual	Rather more than usual	Much more than usual
A6.	Been getting a feeling of tightness of pressure in your head?	Not at all	No more than usual	Rather more than usual	Much more than usual
A7.	Been getting hot or cold spells?	Not at all	No more than usual	Rather more than usual	Much more than usual
B1.	Lost much sleep over worry?	Not at all	No more than usual	Rather more than usual	Much more than usual
B2.	Had difficulty in staying asleep once you are off?	Not at all	No more than usual	Rather more than usual	Much more than usual
B3.	Felt constantly under strain?	Not at all	No more than usual	Rather more than usual	Much more than usual
B4.	Been getting edgy and bad – tempered?	Not at all	No more than usual	Rather more than usual	Much more than usual
B5.	Been getting scared or panicky for no good reason?	Not at all	No more than usual	Rather more than usual	Much more than usual
B6.	Found everything getting on top of you?	Not at all	No more than usual	Rather more than usual	Much more than usual
B7.	Been feeling nervous and strung-up all the time?	Not al all	No more than Usual	Rather more than usual	Much more than usual

HAVE YOU RECENTLY:

C1.	Been managing to keep yourself busy and occupied?	More so than usual	Same as usual	Rather less than usual	Much less than usual
C2.	Felt on the whole you were doing things well?	Quicker than Usual	Same as usual	Longer than usual	Much longer than usual
C3.	Felt on the whole you were doing things well?	Better than Usual	About the same	Less well than usual	Much less well
C4.	Been satisfied with the way you've carried out your tasks?	More Satisfied	About the same as usual	Less satisfied than usual	Much less satisfied
C5.	Felt that you are playing a useful part in things?	More so than Usual	Same as usual	Less useful than usual	Much less useful
C6.	Felt capable of making decisions about things?	More so than Usual	Same as usual	Less so than usual	Much less capable
C7.	Been able to enjoy your normal day-to-day activities?	More so than Usual	Same as usual	Less so than usual	Much less than usual
D1.	Been thinking of yourself as a worthless person?	Not at all	No more than Usual	Rather more than usual	Much more than usual
D2.	Felt that life is entirely hopeless?	Not at all	No more than Usual	Rather more than usual	Much more than usual
D3	Felt that life isn't worth living?	Not at all	No more than usual	Rather more than usual	Much more than usual
D4.	Thought of the possibility that you might do away with yourself?	Definitely not	I don't think so	Has crossed my mind	Definitely have
D5.	Found at times you couldn't do anything because your nerves were too bad?	Not at all	No more than usual	Rather more than usual	Much more than usual
D6.	Found yourself wishing you were dead and away from it all?	Not at all	No more than usual	Rather more than usual	Much more than usual
D7.	Found that the idea of taking your own life kept coming into your mind?	Definitely not	I don't think so	Has crossed my mind	Definitely has

APPENDIX 6 - Parental attitude questionnaire

Parental Attitude Questionnaire

Informing children about their mode of conception

Instructions

Kindly read each question carefully and indicate your answer(s) by ticking the appropriate box(es) and/or circling your chosen option(s). Please feel free to expand on any or all of your answers.

Office Use Only
UK

Ongoing Child and Family Development Study

Please provide all the following *confidential* details

Please fill in the details of the child participating in this study

Date of Birth	Gender	Method of Conception
	*Male/Female	*ICSI/IVF

(*please delete as appropriate)

Are you the child's mother or father*? (*please delete as appropriate)

Please fill in your details below.

Date of Birth	Marital Status*	Nationality

* single/married/separated/living together/widowed/divorced

If you have any other children, please complete the table below
(*please delete as appropriate)

Date of Birth	Gender	Type of Conception	Is this child from the same relationship as the above named child?
	*Male/Female	*ICSI/IVF/natural conception	*Yes/No
	*Male/Female	*ICSI/IVF/natural conception	*Yes/No
	*Male/Female	*ICSI/IVF/natural conception	*Yes/No
	*Male/Female	*ICSI/IVF/natural conception	*Yes/No
	*Male/Female	*ICSI/IVF/natural conception	*Yes/No

Question 1: Whom have you told that your child was conceived after IVF/ICSI?

Please select all of those that apply to you.

- ☐ my set of parents
- ☐ partner's set of parents
- ☐ both sets of parents
- ☐ my/our other children
- ☐ other family members
- ☐ close friends
- ☐ professionals e.g. teachers (please specify _____)
- ☐ we do not mind who knows
- ☐ no – one
- ☐ other (please specify _____)

Further comments:

Question 2: Have you discussed with your child, the 'extra help' you needed when conceiving them? If NO, go to Question 3.

YES

a) How old was your child? _____

b) Before discussing this subject with your child did you read any information, speak with others, attend chat groups or look at websites for ideas on how to approach this subject? Yes/No

If Yes, please specify _____

c) When telling your child, did you use any books, leaflets or any methods for clarification? Yes/No

If Yes, what method(s)? _____

d) Would you have found it easier to tell your child if you had been provided with appropriate child friendly literature on this topic specifically? Yes/No

Further comments:

Question 3: Do you intend to tell your child?

Yes, at some point – go to SECTION A

No, never – go to SECTION B

Undecided – go to SECTION C

SECTION A: I intend to tell my child about their method of conception

a) At what age do you plan to tell your child? ___

b) Have you read any information, talked with others, attended chat groups or looked at websites for ideas on how to approach this subject? Yes/No

If Yes, please specify _____

c) Would it be helpful if we could provide you with some child – friendly literature to help you to explain your child's conception to them? Yes/No

Further comments:

Now go to Question 4.

SECTION B: I do not intend to tell my because ....

Please select all of those that apply to you.

- ☐ as parents we cannot agree on the decision at present
- ☐ I am worried about my child's response
- ☐ I do not want my child to feel different form his/her brother(s) and/or sister(s)
- ☐ I do not want my child to feel different from his/her peer group
- ☐ I do not want my child to reveal how they were conceived to others
- ☐ I am concerned about my child's acceptance within my/my partner's family culture i.e. morals, ethics, beliefs, ideas, religion
- ☐ Other (please specify)

_____

_____

_____

b) **Would you change your mind if we could provide you with appropriate literature?** Yes/No

Now go to Question 4.

SECTION C: I am undecided whether or not to tell my child

a) I am undecided because .....

Please select all of those that apply to you.

- ☐ as parents we cannot agree on the decision at present
- ☐ I am worried about my child's response
- ☐ I think my child is too young
- ☐ I have not discussed basic sex education with my child
- ☐ I do not want my child to feel different from his/her brother(s) and/or sister(s)
- ☐ I do not want my child to feel different from his/her peer group
- ☐ I do not want my child to reveal their method of conception to others
- ☐ I am concerned about my child's acceptance within my/my partner's family's culture i.e. morals, ethics, beliefs, ideas, religion
- ☐ I am unsure how to approach the subject with my child
- ☐ I have not found any appropriate child – friendly literature to use as a guide
- ☐ Other (please specify)

_____

_____

_____

b) Would you tell your child if we could provide you with appropriate literature? Yes/No

Now go to Question 4.

Question 4: Have you found or come across any literature, short films or any other materials addressing the issue of telling children that they were conceived after assisted conception? Yes/No

If Yes, please state the sources of literature that you have found helpful.

_____
_____
_____

If No, have you actively tried to search for literature or any other resources that may be or have been useful? Yes/No

If so, where?

- ☐ Internet
- ☐ Library
- ☐ Bookshops
- ☐ Fertility centre(s) information sources
- ☐ Other (please specify)

_____
_____
_____

Question 5: If literature were to be produced what would be helpful?

Please select all of those that apply.

- ☐ A basic description of natural conception
- ☐ A basic description of the IVF/ICSI process
- ☐ Having pictorial representation(s) of the important points
- ☐ Provision of literature that could be given to the child
- ☐ Provision of literature that you could read to your child

Thank you for taking the time to fill out this questionnaire.

APPENDIX 7 – Genetic Imprinting questionnaire

Genetic Imprinting Questionnaire

(Fertility treatment and Beckwith-Weidemann syndrome)

Dear Parent,

Thank you for taking the time to fill in this questionnaire. The details that you provide will be kept strictly confidential. You will not be identified in any report or publication.

Name of child_____ Date of birth_____

- Information about mother

Name of mother	
Date of birth	
Occupation	

- Information about father

Name of father	
Date of birth	
Occupation	

- Name of condition that your child has been diagnosed with

_____

- Name and address of clinician caring for child

_____

_____

_____

_____

_____

- Could we approach this doctor for further details? Yes / No*
  (*delete if not applicable)
  If yes, please sign the questionnaire at the end.

- Does any one else in your family have this condition? Yes / No*
  (*delete if not applicable)

- If so, what is the relationship of this/ these family members to your child?
  (eg fathers brother (uncle)etc)

_____

_____

_____

- How was your child conceived?

(please tick one or more box as applicable)

☐ Natural conception

☐ Conventional IVF

☐ ICSI (intracytoplasmic sperm injection)

☐ Frozen embryo replacement

☐ Blastocyst transfer

☐ IVF with donor egg

☐ IVF with donor sperm

☐ Other (please state)_____

- How many years did you and your present partner have to wait to conceive?

_____

- What were you told were the cause of your difficulties in getting pregnant?

_____

- Do you have any other children? Yes/No*
  (*delete if not applicable)

- If yes, how many other children do you have and how old are they?

  _____

  _____

  Are they well?_____

  _____

- Did you have any other pregnancies / miscarriages? Yes/No*
  (*delete if not applicable)

- If so, how long did these pregnancies last and were you told what the difficulties were?

_____

_____

_____

_____

- Did you have any problems in early pregnancy- for example, bleeding, fever, and abnormalities on early ultrasound scans? Please state below.

_____

_____

_____

_____

- Please feel free to provide any additional comments or information below

_____

_____

_____

_____

- Please sign below if you are willing for us to contact the doctor caring for your child.

Name of parent_____

Signature_____

REFERENCES

Aase, J. M. (1990) *Diagnostic dysmorphology.* Pleneum Publishing Corporation.

Abidin, R. R. (1990) *Parenting Stress Index Test Manual.* Pediatric Psychology Press.

Abusheikha, N., Salha, O., Sharma, V., Brinsden, P. and Abusheika, N. (2000) Monozygotic twinning and IVF/ICSI treatment: a report of 11 cases and review of literature. *Hum.Reprod.Update.*, **6,** 396-403.

Achenbach, T. M. (1991) *Manual for the Child Behaviour Checklist/4-18.* University of Vermont.

Akande, V. and Murphy, D. J. (2002) Neurological sequelae in in-vitro fertilisation babies. *Lancet*, **360,** 717-718.

Anteby, I., Cohen, E., Anteby, E. and BenEzra, D. (2001) Ocular manifestations in children born after in vitro fertilization. *Arch.Ophthalmol.*, **119,** 1525-1529.

Anthony, S., Buitendijk, S. E., Dorrepaal, C. A., Lindner, K., Braat, D. D. and Den Ouden, A. L. (2002) Congenital malformations in 4224 children conceived after IVF. *Hum.Reprod.*, **17,** 2089-2095.

Barkan, S. E. and Bracken, M. B. (1987) Delayed childbearing: no evidence for increased risk of low birth weight and preterm delivery. *Am.J.Epidemiol.*, **125,** 101-109.

Barnett, S. C. and Riddell, J. S. (2004) Olfactory ensheathing cells (OECs) and the treatment of CNS injury: advantages and possible caveats. *J.Anat.*, **204,** 57-67.

Bene, E. and Anthony J (1978) *Bene-Anthony Family Relations Test.* The NEFR-Nelson Publishing Co. Ltd.

Bergh, T., Ericson, A., Hillensjo, T., Nygren, K. G. and Wennerholm, U. B. (1999) Deliveries and children born after in-vitro fertilisation in Sweden 1982-95: a retrospective cohort study. *Lancet*, **354,** 1579-1585.

Berkowitz, G. S., Skovron, M. L., Lapinski, R. H. and Berkowitz, R. L. (1990) Delayed childbearing and the outcome of pregnancy. *N.Engl.J.Med.*, **322,** 659-664.

Biggs, J. S. (1973) Pregnancy at 40 years and over. *Med.J.Aust.*, **1,** 542-545.

Bonduelle, M., Aytoz, A., Wilikens, A., Buysse, A., Van Assche, E., Devroey, P., Van Steirteghem, A. and Liebaers, I. (1998a) Prospective follow-up study of 1,987 children born after intracytoplasmic sperm injection (ICSI). In *Treatment of Infertility: The New Frontiers.* Communications Media for Education, Inc, pp. 445-461.

Bonduelle, M., Camus, M., De Vos, A., Staessen, C., Tournaye, H., Van Assche, E., Verheyen, G., Devroey, P., Liebaers, I. and Van Steirteghem, A. (1999) Seven years

of intracytoplasmic sperm injection and follow-up of 1987 subsequent children. *Hum.Reprod.*, **14 Suppl 1,** 243-264.

Bonduelle, M., Joris, H., Hofmans, K., Liebaers, I. and Van Steirteghem, A. (1998b) Mental development of 201 ICSI children at 2 years of age. *Lancet North Am.Ed*, **351,** 1553.

Bonduelle, M., Legein, J., Buysse, A., Van Assche, E., Wisanto, A., Devroey, P., Van Steirteghem, A. C. and Liebaers, I. (1996a) Prospective follow-up study of 423 children born after intracytoplasmic sperm injection. *Hum.Reprod.*, **11,** 1558-1564.

Bonduelle, M., Liebaers, I., Deketelaere, V., Derde, M. P., Camus, M., Devroey, P. and Van Steirteghem, A. (2002) Neonatal data on a cohort of 2889 infants born after ICSI (1991--1999) and of 2995 infants born after IVF (1983--1999). *Hum.Reprod.*, **17,** 671-694.

Bonduelle, M., Ponjaert, I., Steirteghem, A. V., Derde, M. P., Devroey, P. and Liebaers, I. (2003) Developmental outcome at 2 years of age for children born after ICSI compared with children born after IVF. *Hum.Reprod.*, **18,** 342-350.

Bonduelle, M., Wennerholm, U. B., Loft, A., Tarlatzis, B. C., Peters, C., Henriet, S., Mau, C., Victorin-Cederquist, A., Van Steirteghem, A., Balaska, A., Emberson, J. R. and Sutcliffe, A. G. (2005) A multi-centre cohort study of the physical health of 5-year-old children conceived after intracytoplasmic sperm injection, in vitro fertilization and natural conception. *Hum.Reprod.*, **20,** 413-419.

Bonduelle, M., Wilikens, A., Buysse, A., Van Assche, E., Devroey, P., Van Steirteghem, A. C. and Liebaers, I. (1998c) A follow-up study of children born after intracytoplasmic sperm injection (ICSI) with epididymal and testicular spermatozoa and after replacement of cryopreserved embryos obtained after ICSI. *Hum.Reprod.*, **13 Suppl 1,** 196-207.

Bonduelle, M., Wilikens, A., Buysse, A., Van Assche, E., Wisanto, A., Devroey, P., Van Steirteghem, A. C. and Liebaers, I. (1996b) Prospective follow-up study of 877 children born after intracytoplasmic sperm injection (ICSI), with ejaculated epididymal and testicular spermatozoa and after replacement of cryopreserved embryos obtained after ICSI. *Hum.Reprod.*, **11 Suppl 4,** 131-155.

Booth, R. T. and Williams, G. L. (1964) Elderly Primigravidae. *Journal of Obstetrics and Gynaecology*, 249-254.

Boreham, R., Airey, C., Erens, B. and Tobin, R. (2002) NHS Patient Survey Programme: General Practice. UK Department of Health. www.dh.gov.uk/PublicationsAndStatistics

Bowen, J. R., Gibson, F. L., Leslie, G. I. and Saunders, D. M. (1998) Medical and developmental outcome at 1 year for children conceived by intracytoplasmic sperm injection. *Lancet,* **351,** 1529-1534.

Brandes, J. M., Scher, A., Itzkovits, J., Thaler, I., Sarid, M. and Gershoni-Baruch, R. (1992) Growth and development of children conceived by in vitro fertilization. *Pediatrics*, **90,** 424-429.

Braverman, A. M., Boxer, A. S., Corson, S. L., Coutifaris, C. and Hendrix, A. (1998) Characteristics and attitudes of parents of children born with the use of assisted reproductive technology. *Fertil.Steril.*, **70,** 860-865.

Brewaeys, A., Golombok, S., Naaktgeboren, N., de Bruyn, J. K. and Van Hall, E. V. (1997) Donor insemination: Dutch parents' opinions about confidentiality and donor anonymity and the emotional adjustment of their children. *Hum.Reprod.*, **12,** 1591-1597.

Briggs, R. and King, T. (1952) Transplantation of Living Nuclei from Blastula Cells into Enucleated Frog Eggs. *Proceedings of the National Academy of Sciences*, **38,** 455-463.

Bruinsma, F., Venn, A., Lancaster, P., Speirs, A. and Healy, D. (2000) Incidence of cancer in children born after in-vitro fertilization. *Hum.Reprod.*, **15,** 604-607.

Cederblad, M., Friberg, B., Ploman, F., Sjoberg, N. O., Stjernqvist, K. and Zackrisson, E. (1996) Intelligence and behaviour in children born after in-vitro fertilization treatment. *Hum.Reprod.*, **11,** 2052-2057.

Chan, A. W., Dominko, T., Luetjens, C. M., Neuber, E., Martinovich, C., Hewitson, L., Simerly, C. R. and Schatten, G. P. (2000) Clonal propagation of primate offspring by embryo splitting. *Science*, **287,** 317-319.

Cnattingius, S., Forman, M. R., Berendes, H. W. and Isotalo, L. (1992) Delayed childbearing and risk of adverse perinatal outcome. A population-based study. *JAMA*, **268,** 886-890.

Cohen, J., Malter, H., Fehilly, C., Wright, G., Elsner, C., Kort, H. and Massey, J. (1988) Implantation of embryos after partial opening of oocyte zona pellucida to facilitate sperm penetration. *Lancet*, **2,** 162.

Cohen, W. R., Newman, L. and Friedman, E. A. (1980) Risk of labor abnormalities with advancing maternal age. *Obstet.Gynecol.*, **55,** 414-416.

Cohn, J. F., Campbell, S. B. and Ross, S. (1991) Infant response in the still-face paradigm at 6 months predicts avoidant and secure attachment at 12 months. *Development and psychopathology*, **3,** 367-376.

Colpin, H., De Munter, A. and Vandemeulebroecke, L. (1998) Parenthood motives in IVF-mothers. *J.Psychosom.Obstet.Gynaecol.*, **19,** 19-27.

Colpin, H., Demyttenaere, K. and Vandemeulebroecke, L. (1995) New reproductive technology and the family: the parent-child relationship following in vitro fertilization. *J.Child Psychol.Psychiatry*, **36,** 1429-1441.

Colpin, H. and Soenen, S. (2002) Parenting and psychosocial development of IVF children: a follow-up study. *Hum.Reprod.*, **17,** 1116-1123.

Cook, R., Vatev, I., Michova, Z. and Golombok, S. (1997) The European study of assisted reproduction families: a comparison of family functioning and child development between Eastern and Western Europe. *J.Psychosom.Obstet.Gynaecol.*, **18,** 203-212.

Cox, G. F., Burger, J., Lip, V., Mau, U. A., Sperling, K., Wu, B. L. and Horsthemke, B. (2002) Intracytoplasmic sperm injection may increase the risk of imprinting defects. *Am.J.Hum.Genet.*, **71,** 162-164.

Cruysberg, J. R., Moll, A. C. and Imhof, S. M. (2002) Bilateral sporadic retinoblastoma in a child born after in vitro fertilization. *Arch.Ophthalmol.*, **120,** 1773-1774.

Cummings, S. M., Savitz, L. A. and Konrad, T. R. (2001) Reported response rates to mailed physician questionnaires. *Health Serv.Res.*, **35,** 1347-1355.

Cummins, J. M. and Jequier, A. M. (1995) Concerns and recommendations for intracytoplasmic sperm injection (ICSI) treatment. *Hum.Reprod.*, **10 Suppl 1,** 138-143.

D'Souza, S. W., Rivlin, E., Buck, P. and Lieberman, B. A. Children conceived by in vitro fertilization. 70-78. 1990. Manchester, Manchester University Press. Clinical IVF forum: current views in assisted reproduction. Mason, P. L. and Lieberman,B.A.

Davies, M. and Norman, R. (2002) Neurological sequelae in in-vitro fertilisation babies. *Lancet*, **360,** 718-719.

de Kretser, D. M. (1995) The potential of intracytoplasmic sperm injection (ICSI) to transmit genetic defects causing male infertility. *Reprod.Fertil.Dev.*, **7,** 137-141.

De Rycke, M., Geuns, E., Manning, M., Lissens, W. and Liebaers, I. (2002a). Assessment of risks related to ICSI – Genomic Imprinting. Unpublished Communication

De Rycke, M., Liebaers, I. and Van Steirteghem, A. (2002b) Epigenetic risks related to assisted reproductive technologies: risk analysis and epigenetic inheritance. *Hum.Reprod.*, **17,** 2487-2494.

DeBaun, M. R., Niemitz, E. L. and Feinberg, A. P. (2003) Association of In Vitro Fertilization with Beckwith-Wiedemann Syndrome and Epigenetic Alterations of LIT1 and H19. *Am.J.Hum.Genet.*, **72,** 156-160.

Devroey, P., Liu, J., Nagy, Z., Goossens, A., Tournaye, H., Camus, M., Van Steirteghem, A. and Silber, S. (1995) Pregnancies after testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermia. *Hum.Reprod.*, **10,** 1457-1460.

Dewey, K. G., Nommsen-Rivers, L. A., Heinig, M. J. and Cohen, R. J. (2003) Risk factors for suboptimal infant breastfeeding behavior, delayed onset of lactation, and excess neonatal weight loss. *Pediatrics*, **112,** 607-619.

Dhont, M., De Sutter, P., Ruyssinck, G., Martens, G. and Bekaert, A. (1999) Perinatal outcome of pregnancies after assisted reproduction: a case-control study. *Am.J.Obstet.Gynecol.*, **181,** 688-695.

DoH (2004). *Health Secretary welcomes new fertility guidance.* UK Department of Health. www.dh.gov.uk/PublicationsAndStatistics/

Doyle, P., Beral, V. and Maconochie, N. (1992) Preterm delivery, low birthweight and small-for-gestational-age in liveborn singleton babies resulting from in-vitro fertilization. *Hum.Reprod.*, **7,** 425-428.

Doyle, P., Bunch, K. J., Beral, V. and Draper, G. J. (1998) Cancer incidence in children conceived with assisted reproduction technology. *Lancet*, **352,** 452-453.

Draper, E. S., Kurinczuk, J. J., Abrams, K. R. and Clarke, M. (1999) Assessment of separate contributions to perinatal mortality of infertility history and treatment: a case-control analysis. *Lancet*, **353,** 1746-1749.

Elder, K. and Dale, B. (2000) *In Vitro Fertilisation.* Cambridge University Press.

Ericson, A. and Kallen, B. (2001) Congenital malformations in infants born after IVF: a population-based study. *Hum.Reprod.*, **16,** 504-509.

Family Planning Association. (2003). *Sex education.* www.fpa.org.uk

Foresta, C. and Ferlin, A. (2001) Offspring conceived by intracytoplasmic sperm injection. *Lancet*, **358,** 1270.
Forman, M. R., Meirik, O. and Berendes, H. W. (1984) Delayed childbearing in Sweden. *JAMA*, **252,** 3135-3139.

Friedler, S., Mashiach, S. and Laufer, N. (1992) Births in Israel resulting from in-vitro fertilization/embryo transfer, 1982-1989: National Registry of the Israeli Association for Fertility Research. *Hum.Reprod.*, **7,** 1159-1163.

Genetic Drift (1995). *Teratogen update*, **12.** www.mostgene.org/gd
Gerris, J. and Van Royen, E. (2000) Avoiding multiple pregnancies in ART: a plea for single embryo transfer. *Hum.Reprod.*, **15,** 1884-1888.

Gibson, F. L., Ungerer, J. A., McMahon, C. A., Leslie, G. I. and Saunders, D. M. (2000a) The mother-child relationship following in vitro fertilisation (IVF): infant attachment, responsivity, and maternal sensitivity. *J.Child Psychol.Psychiatry*, **41,** 1015-1023.

Gibson, F. L., Ungerer, J. A., Tennant, C. C. and Saunders, D. M. (2000b) Parental adjustment and attitudes to parenting after in vitro fertilization. *Fertil.Steril.*, **73,** 565-574.

Gicquel, C., Gaston, V., Mandelbaum, J., Siffroi, J. P., Flahault, A. and Le Bouc, Y. (2003) In vitro fertilization may increase the risk of Beckwith-Wiedemann syndrome related to the abnormal imprinting of the KCN1OT gene. *Am.J.Hum.Genet.*, **72,** 1338-1341.

Gissler, M. and Hemminki, E. (1996) The danger of overmatching in studies of the perinatal mortality and birthweight of infants born after assisted conception. *Eur.J.Obstet.Gynecol.Reprod.Biol.*, **69,** 73-75.

Gissler, M., Malin, S. M. and Hemminki, E. (1995) In-vitro fertilization pregnancies and perinatal health in Finland 1991-1993. *Hum.Reprod.*, **10,** 1856-1861.

Glaser, R. L., Jiang, W., Boyadjiev, S. A., Tran, A. K., Zachary, A. A., Van Maldergem, L., Johnson, D., Walsh, S., Oldridge, M., Wall, S. A., Wilkie, A. O. and Jabs, E. W. (2000) Paternal origin of FGFR2 mutations in sporadic cases of Crouzon syndrome and Pfeiffer syndrome. *Am.J.Hum.Genet.*, **66,** 768-777.

Goldberg, D. P. and Hillier, V. F. (1979) *A scaled version of the General Health Questionnaire.* Cambridge Univ Press., US.

Golombok, S., Brewaeys, A., Cook, R., Giavazzi, M. T., Guerra, D., Mantovani, A., van Hall, E., Crosignani, P. G. and Dexeus, S. (1996) The European study of assisted reproduction families: family functioning and child development. *Hum.Reprod.*, **11,** 2324-2331.

Golombok, S., Brewaeys, A., Giavazzi, M. T., Guerra, D., MacCallum, F. and Rust, J. (2002) The European study of assisted reproduction families: the transition to adolescence. *Hum.Reprod.*, **17,** 830-840.

Golombok, S., MacCallum, F. and Goodman, E. (2001) The "test-tube" generation: parent-child relationships and the psychological well-being of in vitro fertilization children at adolescence. *Child Dev.*, **72,** 599-608.

Greenberger, E. and Goldberg, W. A. (1989) *Work, parenting, and the socialization of children.* American Psychological Assn., Washington.

Greenfeld, D. A., Ort, S. I., Greenfeld, D. G., Jones, E. E. and Olive, D. L. (1996) Attitudes of IVF parents regarding the IVF experience and their children. *J.Assist.Reprod.Genet.*, **13,** 266-274.

Griffin, D. K., Abruzzo, M. A., Millie, E. A., Sheean, L. A., Feingold, E., Sherman, S. L. and Hassold, T. J. (1995) Non-disjunction in human sperm: evidence for an effect of increasing paternal age. *Hum.Mol.Genet.*, **4,** 2227-2232.

Hahn, C. S. (2001) Review: psychosocial well-being of parents and their children born after assisted reproduction. *J.Pediatr.Psychol.*, **26,** 525-538.

Hahn, C. S. and DiPietro, J. A. (2001) In vitro fertilization and the family: quality of parenting, family functioning, and child psychosocial adjustment. *Dev.Psychol.*, **37,** 37-48.

Handelsman, D. J., Conway, A. J., Boylan, L. M. and Turtle, J. R. (1984) Young's syndrome. Obstructive azoospermia and chronic sinopulmonary infections. *N.Engl.J.Med.*, **310,** 3-9.

Hassan, M. A. and Killick, S. R. (2004) Negative lifestyle is associated with a significant reduction in fecundity. *Fertil.Steril.*, **81,** 384-392.

Hassold, T., Abruzzo, M., Adkins, K., Griffin, D., Merrill, M., Millie, E., Saker, D., Shen, J. and Zaragoza, M. (1996) Human aneuploidy: incidence, origin, and etiology. *Environ.Mol.Mutagen.*, **28,** 167-175.

Hazekamp, J., Bergh, C., Wennerholm, U. B., Hovatta, O., Karlstrom, P. O. and Selbing, A. (2000) Avoiding multiple pregnancies in ART: consideration of new strategies. *Hum.Reprod.*, **15,** 1217-1219.

Healy, D. L. and Saunders, K. (2002) Follow-up of children born after in-vitro fertilisation. *Lancet*, **359,** 459-460.

Hershlag, A., Floch, J. A., DeCherney, A. H. and Lavy, G. (1990) Comparison of singleton and multiple pregnancies in in vitro fertilization (IVF) and embryo transfer (ET). *J.In Vitro Fert.Embryo.Transf.*, **7,** 157-159.

Hill, J. R., Roussel, A. J., Cibelli, J. B., Edwards, J. F., Hooper, N. L., Miller, M. W., Thompson, J. A., Looney, C. R., Westhusin, M. E., Robl, J. M. and Stice, S. L. (1999) Clinical and pathologic features of cloned transgenic calves and fetuses (13 case studies). *Theriogenology*, **51,** 1451-1465.

Hjelmstedt, A., Andersson, L., Skoog-Svanberg, A., Bergh, T., Boivin, J. and Collins, A. (1999) Gender differences in psychological reactions to infertility among couples seeking IVF- and ICSI-treatment. *Acta Obstet.Gynecol.Scand.*, **78,** 42-48.

Hoopes, J. L. (1990) Adoption and identity formation. In Brodinsky, D. M. and Scheter, M. D. (eds), *The Psychology of Adoption*. Oxford University Press, Oxford, pp. 144-166.

Hull, M. G., Glazener, C. M., Kelly, N. J., Conway, D. I., Foster, P. A., Hinton, R. A., Coulson, C., Lambert, P. A., Watt, E. M. and Desai, K. M. (1985) Population study of causes, treatment, and outcome of infertility. *Br.Med.J.(Clin.Res.Ed)*, **291,** 1693-1697.

In'tVeld, P., Brandenburg, H., Verhoeff, A., Dhont, M. and Los, F. (1995) Sex chromosomal abnormalities and intracytoplasmic sperm injection. *Lancet*, **346,** 773.

Inhorn, M. C. (1994) Past and Present Theories of Procreation. In *Quest for conception: Gender, Infertility and Egyptian Medical Traditions*. University of Pennsylvania Press, pp. 52-67.

Intercomptex UK (1993). *Pure Tone Audiometer - Owner's Manual.* InterComtex. UK.

Isaksson, R., Gissler, M. and Tiitinen, A. (2002) Obstetric outcome among women with unexplained infertility after IVF: a matched case-control study. *Hum.Reprod.*, **17,** 1755-1761.

Jain, T., Missmer, S. A. and Hornstein, M. D. (2004) Trends in embryo-transfer practice and in outcomes of the use of assisted reproductive technology in the United States. *N.Engl.J.Med.*, **350,** 1639-1645.

Jones, K. L. (1988) Chromosomal Abnormality syndromes. In *Smith's Recognizable Patterns of Human Malformation*. WB Saunders Co., pp. 74-79.

Kane, S. H. (1967) Advancing age and the primigravida. *Obstet.Gynecol.*, **29,** 409-414.

Karpel, M. A. (1980) Family secrets: I. Conceptual and ethical issues in the relational context. II. Ethical and practical considerations in therapeutic management. *Fam.Process*, **19,** 295-306.

Kato, Y., Tani, T. and Tsunoda, Y. (2000) Cloning of calves from various somatic cell types of male and female adult, newborn and fetal cows. *J.Reprod.Fertil.*, **120,** 231-237.

Kent-First, M. G., Kol, S., Muallem, A., Ofir, R., Manor, D., Blazer, S., First, N. and Itskovitz-Eldor, J. (1996) The incidence and possible relevance of Y-linked microdeletions in babies born after intracytoplasmic sperm injection and their infertile fathers. *Mol.Hum.Reprod.*, **2,** 943-950.

Kerin, J. F., Warnes, G. M., Quinn, P. J., Jeffrey, R., Kirby, C., Matthews, C. D., Seamark, R. F. and Cox, L. W. (1983) Incidence of multiple pregnancy after in-vitro fertilisation and embryo transfer. *Lancet*, **2,** 537-540.

Khosla, S., Dean, W., Reik, W. and Feil, R. (2001) Culture of preimplantation embryos and its long-term effects on gene expression and phenotype. *Hum.Reprod.Update.*, **7,** 419-427.

Klemetti, R., Gissler, M. and Hemminki, E. (2002) Comparison of perinatal health of children born from IVF in Finland in the early and late 1990s. *Hum.Reprod.*, **17,** 2192-2198.

Klip, H., Burger, C. W., de Kraker, J. and van Leeuwen, F. E. (2001) Risk of cancer in the offspring of women who underwent ovarian stimulation for IVF. *Hum.Reprod.*, **16,** 2451-2458.

Kobayashi, N., Matsui, I., Tanimura, M., Nagahara, N., Akatsuka, J., Hirayama, T. and Sato, K. (1991) Childhood neuroectodermal tumours and malignant lymphoma after maternal ovulation induction. *Lancet*, **338,** 955.

Koivurova, S., Hartikainen, A. L., Gissler, M., Hemminki, E., Sovio, U. and Jarvelin, M. R. (2002) Neonatal outcome and congenital malformations in children born after in-vitro fertilization. *Hum.Reprod.*, **17,** 1391-1398.

Lansac, J. (1995) Delayed parenting. Is delayed childbearing a good thing? *Hum.Reprod.*, **10,** 1033-1035.

Lashen, H., Afnan, M. and Sharif, K. (1999) A controlled comparison of ovarian response to controlled stimulation in first generation Asian women compared with white Caucasians undergoing in vitro fertilisation. *Br.J.Obstet.Gynaecol.*, **106,** 407-409.

Lerner-Geva, L., Toren, A., Chetrit, A., Modan, B., Mandel, M., Rechavi, G. and Dor, J. (2000) The risk for cancer among children of women who underwent in vitro fertilization. *Cancer*, **88,** 2845-2847.

Leslie, G. I., Bowen, J. R., Arnold, J. D. and Saunders, D. M. (1992) In-vitro fertilisation and neonatal ventilator use in a tertiary perinatal centre. *Med.J.Aust.*, **157,** 165-167.

Leslie, G. I., Gibson, F. L., McMahon, C., Cohen, J., Saunders, D. M. and Tennant, C. (2003) Children conceived using ICSI do not have an increased risk of delayed mental development at 5 years of age. *Hum.Reprod.*, **18,** 2067-2072.

Leslie, G. I., Gibson, F. L., McMahon, C., Tennant, C. and Saunders, D. M. (1998) Infants conceived using in-vitro fertilization do not over-utilize health care resources after the neonatal period. *Hum.Reprod.*, **13,** 2055-2059.

Leviton, A., Stewart, J. E., Allred, E. N., Dammann, O. and Kuban, K. (2002) Neurological sequelae in in-vitro fertilisation babies. *Lancet*, **360,** 718.

LGROW (1998) *Child Growth Measurement Computer program).* Institute of Education and Child Growth Foundation. London

Liebaers, I., Bonduelle, M., Van Assche, E., Devroey, P. and Van Steirteghem, A. (1995) Sex chromosome abnormalities after intracytoplasmic sperm injection. *Lancet*, **346,** 1095.

Love, C. D. and Wallace, E. M. (1996) Pregnancies complicated by placenta praevia: what is appropriate management? *Br.J.Obstet.Gynaecol.*, **103,** 864-867.

Macnab, A. J. and Zouves, C. (1991) Hypospadias after assisted reproduction incorporating in vitro fertilization and gamete intrafallopian transfer. *Fertil.Steril.*, **56,** 918-922.

Maher, E. R., Brueton, L. A., Bowdin, S. C., Luharia, A., Cooper, W., Cole, T. R., Macdonald, F., Sampson, J. R., Barratt, C. L., Reik, W. and Hawkins, M. M. (2003) Beckwith-Wiedemann syndrome and assisted reproduction technology (ART). *J.Med.Genet.*, **40,** 62-64.

Mahmud, G., Lopez, B. A., Yudkin, P., Ledger, W. and Barlow, D. H. (1995) A controlled assessment of the in vitro fertilization performance of British women of Indian origin compared with white women. *Fertil.Steril.*, **64,** 103-106.

Manning, M., Lissens, W., Liebaers, I., Van Steirteghem, A. and Weidner, W. (2001) Imprinting analysis in spermatozoa prepared for intracytoplasmic sperm injection (ICSI). *Int.J.Androl*, **24,** 87-94.

Marsh and Ronner (1996) *The Empty Cradle.* John Hopkins University Press.

McCarthy, D. (1972) *McCarthy Motor Scales of Children's Abilities.* The Psychological Corporation, New York

McDevitt, S. C. and Carey, W. B. (1978) *The measurement of temperament in 3-7 year old children*. Blackwell Publishers, Oxford.

McEvoy, T. G., Sinclair, K. D., Broadbent, P. J., Goodhand, K. L. and Robinson, J. J. (1998) Post-natal growth and development of Simmental calves derived from in vivo or in vitro embryos. *Reprod.Fertil.Dev.*, **10,** 459-464.

McGuire, J. and Earls, F. (1993) *Exploring the reliability of measures of family relations, parental attitudes, and parent-child relations in a disadvantaged minority population*. Allen Press., US.

McIntosh, G. C., Olshan, A. F. and Baird, P. A. (1995) Paternal age and the risk of birth defects in offspring. *Epidemiology*, **6,** 282-288.

McKibbin, M. and Dabbs, T. R. (1996) Assisted conception and retinopathy of prematurity. *Eye*, **10 (4),** 476-478.

McMahon, C. A., Ungerer, J. A., Beaurepaire, J., Tennant, C. and Saunders, D. (1997a) Anxiety during pregnancy and fetal attachment after in-vitro fertilization conception. *Hum.Reprod.*, **12,** 176-182.

McMahon, C. A., Ungerer, J. A., Tennant, C. and Saunders, D. (1997b) Psychosocial adjustment and the quality of the mother-child relationship at four months postpartum after conception by in vitro fertilization. *Fertil.Steril.*, **68,** 492-500.

McWhinnie, A. (1996) Outcome for families created by assisted conception programmes. *J.Assist.Reprod.Genet.*, **13,** 363-365.

Meschede, D. and Horst, J. (1997) Sex chromosomal anomalies in pregnancies conceived through intracytoplasmic sperm injection: a case for genetic counselling. *Hum.Reprod.*, **12,** 1125-1127.

Moll, A. C., Imhof, S. M., Cruysberg, J. R., Schouten-van Meeteren, A. Y., Boers, M. and van Leeuwen, F. E. (2003) Incidence of retinoblastoma in children born after in-vitro fertilisation. *Lancet*, **361,** 309-310.

Mollard, R., Denham, M. and Trounson, A. (2002) Technical advances and pitfalls on the way to human cloning. *Differentiation*, **70,** 1-9.

Montgomery, T. R., Aiello, F., Adelman, R. D., Wasylyshyn, N., Andrews, M. C., Brazelton, T. B., Jones, G. S. and Jones, H. W., Jr. (1999) The psychological status at school age of children conceived by in-vitro fertilization. *Hum.Reprod.*, **14,** 2162-2165.

Morin, N. C., Wirth, F. H., Johnson, D. H., Frank, L. M., Presburg, H. J., Van, d. W., V, Chee, E. M. and Mills, J. L. (1989) Congenital malformations and psychosocial development in children conceived by in vitro fertilization. *J.Pediatr.*, **115,** 222-227.

MRC (1990) Births in Great Britain resulting from assisted conception, 1978-87. MRC Working Party on Children Conceived by In Vitro Fertilisation. *BMJ*, **300,** 1229-1233.

Mushin, D. N., Barreda-Hanson, M. C. and Spensley, J. C. (1986) In vitro fertilization children: early psychosocial development. *J.In Vitro Fert.Embryo.Transf.*, **3,** 247-252.

Ng, S. C., Bongso, A., Ratnam, S. S., Sathananthan, H., Chan, C. L., Wong, P. C., Hagglund, L., Anandakumar, C., Wong, Y. C. and Goh, V. H. (1988) Pregnancy after transfer of sperm under zona. *Lancet*, **2,** 790.

Olivennes, F. (2003) Patient-friendly ovarian stimulation. *Reprod.Biomed.Online.*, **7,** 30-34.

Olivennes, F., Kerbrat, V., Rufat, P., Blanchet, V., Fanchin, R. and Frydman, R. (1997) Follow-up of a cohort of 422 children aged 6 to 13 years conceived by in vitro fertilization. *Fertil.Steril.*, **67,** 284-289.

Olivennes, F., Mannaerts, B., Struijs, M., Bonduelle, M. and Devroey, P. (2001) Perinatal outcome of pregnancy after GnRH antagonist (ganirelix) treatment during ovarian stimulation for conventional IVF or ICSI: a preliminary report. *Hum.Reprod.*, **16,** 1588-1591.

Olivennes, F., Rufat, P., Andre, B., Pourade, A., Quiros, M. C. and Frydman, R. (1993) The increased risk of complication observed in singleton pregnancies resulting from in-vitro fertilization (IVF) does not seem to be related to the IVF method itself. *Hum.Reprod.*, **8,** 1297-1300.

Orioli, I. M., Castilla, E. E., Scarano, G. and Mastroiacovo, P. (1995) Effect of paternal age in achondroplasia, thanatophoric dysplasia, and osteogenesis imperfecta. *Am.J.Med.Genet.*, **59,** 209-217.

Page, D. C., Silber, S. and Brown, L. G. (1999) Men with infertility caused by AZFc deletion can produce sons by intracytoplasmic sperm injection, but are likely to transmit the deletion and infertility. *Hum.Reprod.*, **14,** 1722-1726.

Palermo, G., Joris, H., Derde, M. P., Camus, M., Devroey, P. and Van Steirteghem, A. (1993) Sperm characteristics and outcome of human assisted fertilization by subzonal insemination and intracytoplasmic sperm injection. *Fertil.Steril.*, **59,** 826-835.

Palermo, G., Joris, H., Devroey, P. and Van Steirteghem, A. C. (1992) Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet*, **340,** 17-18.

Peters, C., Kantaris, X., Barnes, J. and Sutcliffe, A. G. (2005) Parental attitudes towards disclosure of the mode of conception to their child conceived by in-vitro fertilisation . *Fertil.Steril.*.

Petersen, K., Hornnes, P. J., Ellingsen, S., Jensen, F., Brocks, V., Starup, J., Jacobsen, J. R. and Andersen, A. N. (1995) Perinatal outcome after in vitro fertilisation. *Acta Obstet.Gynecol.Scand.*, **74,** 129-131.

Pharoah, P. O. (2002) Neurological outcome in twins. *Semin.Neonatol.*, **7,** 223-230.

Ponjaert-Kristoffersen, Bonduelle, M., Barnes, J., Nekkebroeck, J., Loft, A., Wennerholm, U. B., Tarlatzis, B. C., Peters, C., Hagberg, B. S., Berner, A. and Sutcliffe, A. G. (2005) International collaborative study of intracytoplasmic sperm injection-conceived, in vitro fertilization-conceived and naturally conceived 5-year-old child outcomes; Cognitive and motor assessments . *Pediatrics*.

Poulakis, V., Witzsch, U., Diehl, W., de Vries, R., Becht, E. and Trotnow, S. (2001) Birth of two infants with normal karyotype after intracytoplasmic injection of sperm obtained by testicular extraction from two men with nonmosaic Klinefelter's syndrome. *Fertil.Steril.*, **76,** 1060-1062.

Qualifications and Curriculum Authority. (2003) *Science at key stage 3 (Year 7) Unit 7B: Reproduction*. Department for Education and Skills www.standards.dfes.gov.uk

Raoul-Duval, A., Bertrand-Servais, M. and Frydman, R. (1993) Comparative prospective study of the psychological development of children born by in vitro fertilization and their mothers. *J.Psychosom.Obstet.Gynaecol.*, **14,** 117-126.
Ratcliffe, S. (1999) Long-term outcome in children of sex chromosome abnormalities. *Arch.Dis.Child*, **80,** 192-195.

Reeve, A. E., Becroft, D. M., Morison, I. M. and Fukuzawa, R. (2002) Insulin-like growth factor-II imprinting in cancer. *Lancet*, **359,** 2050-2051.

Saunders, K., Spensley, J., Munro, J. and Halasz, G. (1996) Growth and physical outcome of children conceived by in vitro fertilization. *Pediatrics*, **97,** 688-692.

Sbracia, M., Baldi, M., Cao, D., Sandrelli, A., Chiandetti, A., Poverini, R. and Aragona, C. (2002) Preferential location of sex chromosomes, their aneuploidy in human sperm, and their role in determining sex chromosome aneuploidy in embryos after ICSI. *Hum.Reprod.*, **17,** 320-324.

Schieve, L. A., Meikle, S. F., Ferre, C., Peterson, H. B., Jeng, G. and Wilcox, L. S. (2002) Low and very low birth weight in infants conceived with use of assisted reproductive technology. *N.Engl.J.Med.*, **346,** 731-737.

Sharara, F. I. and McClamrock, H. D. (2000) Differences in in vitro fertilization (IVF) outcome between white and black women in an inner-city, university-based IVF program. *Fertil.Steril.*, **73,** 1170-1173.

Shiels, P. G., Kind, A. J., Campbell, K. H., Waddington, D., Wilmut, I., Colman, A. and Schnieke, A. E. (1999) Analysis of telomere lengths in cloned sheep. *Nature*, **399,** 316-317.

Silber, S. J., Nagy, Z. P., Liu, J., Godoy, H., Devroey, P. and Van Steirteghem, A. C. (1994) Conventional in-vitro fertilization versus intracytoplasmic sperm injection for patients requiring microsurgical sperm aspiration. *Hum.Reprod.*, **9,** 1705-1709.

Silber, S. J., Van Steirteghem, A. C., Liu, J., Nagy, Z., Tournaye, H. and Devroey, P. (1995) High fertilization and pregnancy rate after intracytoplasmic sperm injection with spermatozoa obtained from testicle biopsy. *Hum.Reprod.*, **10,** 148-152.

Simerly, C., Dominko, T., Navara, C., Payne, C., Capuano, S., Gosman, G., Chong, K. Y., Takahashi, D., Chace, C., Compton, D., Hewitson, L. and Schatten, G. (2003) Molecular correlates of primate nuclear transfer failures. *Science*, **300,** 297.

Simpson, J. L. (1996) Registration of congenital anomalies in ART populations: pitfalls. *Hum.Reprod.*, **11 Suppl 4,** 81-88.

Spanier, G. B. (1976) *Measuring dyadic adjustment: New scales for assessing the quality of marriage and similar dyads*. Allen Press, US.

Steptoe, P. C. and Edwards, R. G. (1978) Birth after the reimplantation of a human embryo. *Lancet*, **2,** 366.

StereoTest (1994). *Titmus Stereotest Manual.* Stereo Optical Co., Inc. Chicago.

Stromberg, B., Dahlquist, G., Ericson, A., Finnstrom, O., Koster, M. and Stjernqvist, K. (2002) Neurological sequelae in children born after in-vitro fertilisation: a population-based study. *Lancet*, **359,** 461-465.

Sutcliffe, A. G., D'Souza, S. W., Cadman, J., Richards, B., McKinlay, I. A. and Lieberman, B. (1995) Outcome in children from cryopreserved embryos. *Arch.Dis.Child*, **72,** 290-293.

Sutcliffe, A. G., Saunders, K., McLachlan, R., Taylor, B., Edwards, P., Grudzinskas, G., Leiberman, B. and Thornton, S. (2003) A retrospective case-control study of developmental and other outcomes in a cohort of Australian children conceived by intracytoplasmic sperm injection compared with a similar group in the United Kingdom. *Fertil.Steril.*, **79,** 512-516.

Sutcliffe, A. G., Taylor, B., Li, J., Thornton, S., Grudzinskas, J. G. and Lieberman, B. A. (1999) Children born after intracytoplasmic sperm injection: population control study. *BMJ*, **318,** 704-705.

Sutcliffe, A. G., Taylor, B., Saunders, K., Thornton, S., Lieberman, B. A. and Grudzinskas, J. G. (2001) Outcome in the second year of life after in-vitro fertilisation by intracytoplasmic sperm injection: a UK case-control study. *Lancet*, **357,** 2080-2084.

Sutcliffe, A. G., Edwards, P. R., Beeson, C. and Barnes, J. (2004) Comparing Parents' Perceptions IVF Conceived Children's Behavior with Naturally Conceived Children. *Infant Mental Health Journal*, **75,** 163-170.

Tallo, C. P., Vohr, B., Oh, W., Rubin, L. P., Seifer, D. B. and Haning, R. V., Jr. (1995) Maternal and neonatal morbidity associated with in vitro fertilization. *J.Pediatr.*, **127,** 794-800.

Tan, S. L., Doyle, P., Campbell, S., Beral, V., Rizk, B., Brinsden, P., Mason, B. and Edwards, R. G. (1992) Obstetric outcome of in vitro fertilization pregnancies compared with normally conceived pregnancies. *Am.J.Obstet.Gynecol.*, **167,** 778-784.

Tanbo (2002) Systematic review of children born from ICSI. *Norwegian Centre for Health Technology and Assessment.* www.oslo.sintef.no

Templeton, A. (2000) Avoiding multiple pregnancies in ART: replace as many embryos as you like-one at a time. *Hum.Reprod.*, **15,** 1662.

Tiepolo, L. and Zuffardi, O. (1976) Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. *Hum.Genet.*, **34,** 119-124.

Tournaye, H., Devroey, P., Liu, J., Nagy, Z., Lissens, W. and Van Steirteghem, A. (1994) Microsurgical epididymal sperm aspiration and intracytoplasmic sperm injection: a new effective approach to infertility as a result of congenital bilateral absence of the vas deferens. *Fertil.Steril.*, **61,** 1045-1051.

Trounson, A. O., Leeton, J. F., Wood, C., Webb, J. and Wood, J. (1981) Pregnancies in humans by fertilization in vitro and embryo transfer in the controlled ovulatory cycle. *Science*, **212,** 681-682.

van Balen, F. (1996) Child-rearing following in vitro fertilization. *J.Child Psychol.Psychiatry*, **37,** 687-693.

van Balen, F., Naaktgeboren, N. and Trimbos-Kemper, T. C. (1996) In-vitro fertilization: the experience of treatment, pregnancy and delivery. *Hum.Reprod.*, **11,** 95-98.

van Balen, F. and Trimbos-Kemper, T. C. (1995) Involuntarily childless couples: their desire to have children and their motives. *J Psychosom Obstet Gynecol*, **16,** 137-144.

Van Steirteghem, A. C., Liu, J., Joris, H., Nagy, Z., Janssenswillen, C., Tournaye, H., Derde, M. P., Van Assche, E. and Devroey, P. (1993a) Higher success rate by intracytoplasmic sperm injection than by subzonal insemination. Report of a second series of 300 consecutive treatment cycles. *Hum.Reprod.*, **8,** 1055-1060.

Van Steirteghem, A. C., Nagy, Z., Joris, H., Liu, J., Staessen, C., Smitz, J., Wisanto, A. and Devroey, P. (1993b) High fertilization and implantation rates after intracytoplasmic sperm injection. *Hum.Reprod.*, **8,** 1061-1066.

Verlaenen, H., Cammu, H., Derde, M. P. and Amy, J. J. (1995) Singleton pregnancy after in vitro fertilization: expectations and outcome. *Obstet.Gynecol.*, **86,** 906-910.

Vilska, S., Tiitinen, A., Hyden-Granskog, C. and Hovatta, O. (1999) Elective transfer of one embryo results in an acceptable pregnancy rate and eliminates the risk of multiple birth. *Hum.Reprod.*, **14,** 2392-2395.

Viville, S., Mollard, R., Bach, M. L., Falquet, C., Gerlinger, P. and Warter, S. (2000) Do morphological anomalies reflect chromosomal aneuploidies?: case report. *Hum.Reprod.*, **15,** 2563-2566.

Vogt, P. H., Edelmann, A., Hirschmann, P. and Kohler, M. R. (1995) The azoospermia factor (AZF) of the human Y chromosome in Yq11: function and analysis in spermatogenesis. *Reprod.Fertil.Dev.*, **7,** 685-693.

Watts, P. and Adams, G. G. (2000) In vitro fertilisation and stage 3 retinopathy of prematurity. *Eye*, **14 (3),** 330-333.

Wennerholm, U. B. and Bergh, C. (2000) Obstetric outcome and follow-up of children born after in vitro fertilization (IVF). *Hum.Fertil.*, **3,** 52-64.

Wennerholm, U. B., Bergh, C., Hamberger, L., Lundin, K., Nilsson, L., Wikland, M. and Kallen, B. (2000) Incidence of congenital malformations in children born after ICSI. *Hum.Reprod.*, **15,** 944-948.

Weschler, D. (1990) *Wechsler Preschool and Primary Scale of Intelligence – Revised (WPPSI-R^{UK})*. The Psychological Corporation Ltd. New York.

Westergaard, H. B., Johansen, A. M., Erb, K. and Andersen, A. N. (2000) Danish National IVF Registry 1994 and 1995. Treatment, pregnancy outcome and complications during pregnancy. *Acta Obstet.Gynecol.Scand.*, **79,** 384-389.

White, L., Giri, N., Vowels, M. R. and Lancaster, P. A. (1990) Neuroectodermal tumours in children born after assisted conception. *Lancet*, **336,** 1577.

WHO (1992) *Manual of International Statistical Classification of Diseases, Injuries and Causes of Death (ICD)*. World Health Organisation.

Williams, N. (2002) Dolly clouds cloning hopes. *Curr.Biol.*, **12,** R79-R80.

Wilmut, I., Schnieke, A. E., McWhir, J., Kind, A. J. and Campbell, K. H. (1997) Viable offspring derived from fetal and adult mammalian cells. *Nature*, **385,** 810-813.

Wisanto, A., Magnus, M., Bonduelle, M., Liu, J., Camus, M., Tournaye, H., Liebaers, I., Van Steirteghem, A. C. and Devroey, P. (1995) Obstetric outcome of 424 pregnancies after intracytoplasmic sperm injection. *Hum.Reprod.*, **10,** 2713-2718.

Young, L. E., Sinclair, K. D. and Wilmut, I. (1998) Large offspring syndrome in cattle and sheep. *Rev.Reprod.*, **3,** 155-163.

Yovich, J. L., Parry, T. S., French, N. P. and Grauaug, A. A. (1986) Developmental assessment of twenty in vitro fertilization (IVF) infants at their first birthday. *J.In Vitro Fert.Embryo.Transf.*, **3,** 253-257.

Zech, H., Vanderzwalmen, P., Prapas, Y., Lejeune, B., Duba, E. and Schoysman, R. (2000) Congenital malformations after intracytoplasmic injection of spermatids. *Hum.Reprod.*, **15,** 969-971.

PUBLICATIONS AND PRESENTATIONS

Publications

Peters, C., Kantaris, X.,Barnes, J., Sutcliffe, A.G. (2005) Parental attitudes towards disclosure of the mode of conception to their child conceived by in-vitro fertilisation *Fertility and Sterility,* 83 (4), 914-919

Bonduelle M, Wennerholm U-B, Loft A, Tarlatzis BC, Peters C, Henriet S, Mau C, Victorin-Cederquist Å, Van Steirteghem A, Balaska A, Emberson J R and Sutcliffe A G (2005) A multi-centre cohort study of the physical health of five-year-old children conceived after intracytoplasmic sperm injection, in vitro fertilisation and natural conception. *Human Reproduction*, 20 (2), 413-419

Ponjaert-Kristoffersen I, Bonduelle M, Barnes J, Nekkebroeck J, Loft A, Wennerholm U-B, Tarlatzis BC, Peters C, Hagberg BS, Berner A, Sutcliffe AG (2005) International collaborative study of intracytoplasmic sperm injection-conceived, in vitro fertilization-conceived and naturally conceived 5-year-old child outcomes; Cognitive and motor assessments *Pediatrics,* 115 (3), e283-9

Presentations

Peters, CJ; Chrysostomou, X; Edwards, P; Sutcliffe, AG. A national study of five year old ICSI conceived children compared with naturally conceived and IVF conceived controls. *Royal Society of Medicine: Paediatrics – International meeting (Oslo) 2003.*

Peters, CJ; Chrysostomou, X; Edwards, P; Barnes, J; Sutcliffe AG An investigation of parental attitudes concerning mode of conception and informing the in-vitro fertilisation (IVF) conceived child *British Fertility Society Annual Meeting, Aberdeen 2003*

Peters CJ, Edwards P, Sutcliffe AG. An interim analysis of UK data from a national prospective study of five year old children (and their families) born after Intracytoplasmic Sperm Injection or natural conception *RCPCH Annual Spring Meeting 2002*